Hilton: Bird's Eye Spot Leaf Disease of the Hevea Rubber Tree caused by Helminthosporium Heveae Petch

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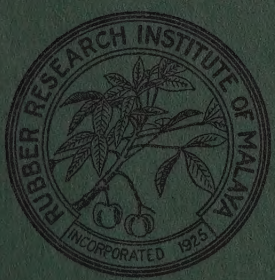


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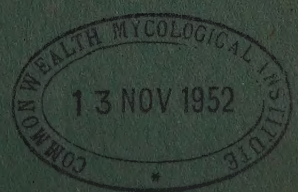

# BIRD'S EYE SPOT LEAF DISEASE OF THE HEVEA RUBBER TREE CAUSED BY HELMINTHOSPORIUM HEVEAE PETCH

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# I. INTRODUCTION

The genus *Helminthosporium* is wellknown as a parasite of cereals and other cultivated grasses both in temperate and tropical regions. As a parasite of broadleaved crops it is mentioned only casually in the literature and does not seem to have called for any specific control measures. A search of the literature reveals only *H.lycopersici*, *H.helianthi*, and *H.gossypii* as recognised *Helminthosporium* leaf diseases of Dicotyledons. The present investigation was called for by the realisation that the confidence of early investigators that Bird's Eye Spot disease of rubber was not serious, and that if it became so it would readily succumb to routine copper or sulphur treatments, was by no means justified.

This investigation has been carried out largely over the two years from September 1948 to August 1950, abundant material being available in 200 acres of nursery at the Rubber Research Institute Experiment Station, Sungei Buloh, Selangor.

It soon became evident that the age of the host and stage of development of host organs were of considerable importance in determining the course of the disease. Accordingly an attempt is made in this paper to use terms referring to age with precision. Plants are referred to as juvenile or adult, a tree that has commenced the annual leaf fall that starts about 4-5 years of age ('wintering'), being considered as adult. Flushes are mature or immature according to whether the leaves of which they are composed have reached a horizontal position or not. Leaves are young or old according to the length of time they have been members of a mature flush; before the flush becomes mature they are called 'immature' leaves. Juvenile and adult trees may be either seedlings or buddings, and are specified as such.

# II. THE DISEASE

## GEOGRAPHICAL DISTRIBUTION

### Asia

The first definite record of the disease is from Ceylon, being reported by Petch in the *Tropical Agriculturist* for June, 1905, and first properly described in the *Annals of the Peradeniya Botanic Gardens*, 1906. However, it is likely that it was first recognised as troublesome in 1904 when specimens from the Malay Peninsula were sent to H. N. Ridley for examination (Ridley, 1904). The next year the same disease. still unidentified, was again received from the Peninsula and found by Ridley himself in Singapore (Ridley, 1905*a*). Several more specimens were sent to Ridley and he despatched a sample of leaves to G. Massee at Kew for identification. Massee reported 'the pale blotches on the leaves are caused by some species of *Cercospora*, but the

absence of fruit prevents specific identification' (Ridley 1905*b*). The next year Ridley received diseased leaves from Ceylon sent by Petch who intended to name the causal fungus *Helminthosporium heveae*. Ridley recognised the disease as being the same as the common Malayan leaf spotting fungus (Ridley 1906), and later in the year Petch's description was published in the *Agricultural Bulletin of the Straits Settlements* (Petch 1906*b*).

Ridley's identification must have been overlooked by Bancroft in 1911 as Bancroft states, in the *Agricultural Bulletin* for that year, that there was no record of its occurrence. The records of Chipp (1920*a*, 1920*b*) appear to have been derived from Ridley's reports, and give no indication that Chipp observed the disease himself. Sharples (1923) reports a *Helminthosporium* on rubber that he records as a new species. It is not until 1927 that the disease was clearly recognised and discussed (Sharples, 1927). Since 1927 there have been yearly references to *H.heveae* in Malaya.

During the twenty years it was overlooked in Malaya it was reported from Java by Rutgers and Arens in 1914, from Sumatra by La Rue in 1922, and from South India by Butler in 1918. Butler seems to have been aware of Ridley's record of 1906 for he mentions *H.heveae* as occurring in Malaya, as well as in Java and South India. Steinmann (1925) reports the disease as very rare in Java, but it has been recorded as widespread in Sumatra since 1921, and, according to La Rue, must have been in evidence there several years before that date.

There is no record for Siam, but Barat (1930) gives the first record for Indo China as being in the year 1928.

Burma, too, provides no record, but the disease is certainly present in the Andaman Islands, being first reported there by Mitra (1929).

The disease was described as wellknown in the Philippine Islands by Reinking (1918).

The disease has been observed in Sarawak, where it was widespread in 1949, and in British North Borneo, 1951.

AMERICA

The distribution of the disease in tropical America, to which the genus *Hevea* is indigenous, is obviously of great interest in any consideration of the country of origin of *Helminthosporium heveae*. The only record for Brazil itself is that of Weir in 1926. In 1915 Vincens had found symptoms very like those of Bird's Eye Spot, but was unable to confirm the disease by finding the spores.

Cultivation of *Hevea* has spread to parts of tropical America other than those to which it is indigenous. Bird's Eye Spot has appeared in some of these areas. In Nicaragua it was found and studied by Kevorkian in 1943. The *Plant Disease Reporter* reported it from Mexico in 1947 (Martin 1947). Correspondents in Honduras and Guatemala confirmed its presence in these countries in 1950.

No records can be found for the other American countries in which rubber is grown.

### Africa

Despite the quite large areas of *Hevea* rubber in Africa, very little information is available concerning disease distribution there. Only one published record has been found, that of Staner (1941) from the Belgian Congo. Correspondence has confirmed its presence in Nigeria in 1951.

## ECONOMIC IMPORTANCE

As early as 1906 Ridley mentions the disease as occurring throughout Malaya, but it did not call for any alarm. The increasing amount of attention paid to the disease over the past twenty years may well be due to the change in planting procedure rather than to increased prevalence or virulence. In the days when the seedling would be grown straight through to a tree, slight retardation in the first twelve months was of little importance. The disease did not kill the plant, but brought about general weakening and defoliation. After one or two years the plants grew out of the disease altogether. However, with the modern demand for seedling stocks on which high yielding clones are to be budded, it becomes of considerable importance for sufficient girth to be attained within the twelve month period; otherwise planting programmes may be disrupted and the early development of new plantings seriously impaired (Altson 1950*a*).

Kevorkian (1948) reports that although the importance of *Helminthosporium heveae* in Nicaragua is insignificant compared with South American Leaf Blight, it is in this effect of interfering with the budding programme that its importance lies. He found that the percentage of budding 'takes' was four times greater for slightly infected plants than for heavily infected plants.

## SYMPTOMS

The name 'Bird's Eye Spot' describes the symptom which was considered most prominent by the earlier writers. It is used for the first time in Petch's textbook of 1911. His, the first, description of the spots (1906) is as follows:

'Minute purple spots which afterwards become white, semi-transparent, circular, 1-5 mm diameter surrounded by a brown line'.

Although subsequent authors have copied this description it is by no means always true that the disease has this appearance, and the description is misleading in that the larger spots are not necessarily a later stage of the smaller. The following description of the symptoms takes into account the age of the leaf at the time of infection:

### Leaf under one fortnight old

On a very young leaf the disease first appears as small dark brown spots about 0.2 mm in diameter. They have a water soaked appearance contrasting with the shining red brown healthy leaflet. The rapidly growing leaf becomes severely distorted in the vicinity of the lesion, a distortion which will persist

into maturity. The lesion itself, however, does not enlarge and produces only a few spores.

Normally a number of spores land on the newly unfolded lamina, and in that case the water soaked lesions tend to unite and a very vigorous growth of the fungus results. This growth is at the expense of the leaflet, the organisation of which completely breaks down. The infected area quickly becomes covered with dark mycelium and masses of spores, giving a black appearance. The leaflet and petiole then drop, and the whole of the new flush may be defoliated in this way. Such an infection may be called 'rampant'.

A severe reaction to such defoliation appears in the symptom known as 'sausage-top' in which the naked upper portion of the stem becomes visibly swollen (Plate 1). No cases of such severe defoliation by *Helminthosporium* alone are known to have actually led to death.

Leaf 2-3 Weeks old

The earliest stage of the disease on a leaf from which most of the anthocyanin has disappeared and in which the soft shining cuticle has begun to harden and become dull, consists of a minute yellow spot readily visible with the aid of a hand lens. Within 24 hours this will have been surrounded by a narrow black margin, which appears as though drawn with a fine pencil. The margin may delimit an area 1-3 mm in diameter, depending on the exact age at which the leaf was infected. Two days later the lesion appears as a straw coloured spot, the tissue enclosed by the margin having died, and within another two days the straw colour has turned to white. It is at this stage that sporing commences.

The spores themselves are very characteristic, appearing under the hand lens as dark brown, shining, elongated objects, often as much as 0.2 mm in length. They lie on the clear area on the underside of the leaf lesion. When abundant they are readily visible to the naked eye as dark chocolate brown patches on the underside of the leaf.

Only later, some 3 weeks after infection and on a leaf which may be about 5 weeks old, do the symptoms appear that are always described as being characteristic for *Helminthosporium heveae*, and from which the name 'Bird's Eye Spot' has been derived. By this time the white centre consists only of the upper and lower cuticles with fragments of hyphae in between. The margin has become raised and is of a distinct red brown colour. The whole is surrounded by a yellow halo merging into the normal green leaf tissue. These spots are usually 2-3 mm in diameter but may be as much as 5 mm. On the underside are a few conidiophores and perhaps 20-30 spores.

The centre of the spot, consisting only of cuticle, is translucent and delicate. It is readily torn, and in older leaves becomes pierced, resulting in a 'shot-hole' effect which seems to have been described as a separate disease by the earlier writers. Some explanation for this description is provided by the fact that eventually the *Helminthosporium* spores disappear

completely and saprophytes come in. A very common saprophytic species is *Cladosporium herbarum*, which may be the organism that was held responsible for the 'shothole leaf disease' attributed to *Scolecotrichum heveae*, the latter being a fungus not unlike *Cladosporium* from its description (Vincens 1915). Small black fruit bodies may be embedded in, or sandwiched between, the upper and lower cuticles. These belong to three or four wellknown genera, and are discussed further on page .....

### Leaf over 3 Weeks old

The earliest stage of infection on a leaf that has passed through the large flaccid green stage and is approaching maturity is similar to that on the young bronze leaf in being discreet and dark brown. But it remains discreet and becomes surrounded by a heavy tannin deposit round which is a narrow halo of yellow leaf tissue. Sporing is slight, or may not occur at all.

The net result of the above variations in type of infection is that any single leaf does not conform to any one set of symptoms, but is a combination of two or three. Sporing is so rapid, particularly in the early stages (when it may occur within 3 or 4 days), that multiple infection is the rule. The tip of the leaf may show a rampant type of infection and be shrivelled, and the rest of the leaf show more typical and bordered lesions produced by spores originating from the infection at the tip. The leaf surface between the large lesions may be occupied by numerous smaller lesions, again derived from spores coming from the same leaf. At this time other fungi, particularly *Gloeosporium*, may be responsible for other small spots. Mites (*Hemitarsonemus latus*) may also cause the leaf to look even more worn and unsightly.

Petioles are frequently attacked, showing dark elongated flecks which sometimes spore. Lesions on the midrib of the leaf and on the petiole are similar in appearance.

At the stage of rapid elongation of the stem, which occurs between emergence from the bud and unfolding of the new flush, the young stem is susceptible to attack but only localised, non-sporing, dark, elongated lesions appear to develop.

### Assessment of Disease

It can be appreciated that mere counting of the lesions gives no idea of the severity of the disease, as 4 or 5 lesions that combine and cause leaf fall in the early stage are much more 'severe' than 40 or 50 lesions on a leaf approaching maturity, where they will be enclosed and have no chance to develop further.

It is clear from the above discussion of symptoms that any assessment of disease should be made on leaves that have reached maturity, after which no further increase in disease will occur, and before secondary organisms come in and mask the effect of the *Helminthosporium*. In practice the penultimate flush has been chosen for this purpose.

# III. CAUSAL ORGANISM

## MORPHOLOGY

### Dimensions

Petch's original description of *Helminthosporium heveae* is as follows: Hyphis sparsis, olivaceis, simplicibus, 80 - 200$\mu$ long.; Conidiis cymbiformibus, 8 - 11 septatis, brunneis, 100 - 120$\mu$ x 15 - 18$\mu$.

This was given by Petch for the Ceylon fungus. Petch illustrates the conidiophore and conidium on page 5 of his *Diseases of the Tea Bush*.

The next descriptions of the fungus are those of La Rue (1923) from Sumatra. He describes the newly formed spores as hyaline, curved, and non-septate. He noted that they darken and become vacuolate later, becoming finally quite dark with rather evenly spaced septa. La Rue remarks that the vacuolate condition sometimes persists. The spores studied by La Rue only differ from those of Petch in being smaller in size. A comparison between the Ceylon, Sumatran, and Malayan measurements is given in Table I below:

TABLE I

| Source | Dimensions | Ceylon (Petch) | Sumatra (La Rue) | Malaya (Author) |
|---|---|---|---|---|
| From plant | Length of spores | 100-200$\mu$ | 38-114$\mu$ | 54-150$\mu$ |
| | Width of spores | 15-18$\mu$ | 11.9-17.0$\mu$ | 10-18$\mu$ |
| | Number of septa | 8-11 | 1-10 | 8-11 |
| | Length of conidiophores | 80-200$\mu$ | — | 100-200$\mu$ |
| From culture | Length of spores | not reported | 22.8-83.6$\mu$ | 52-110$\mu$ |
| | Width of spores | | not reported | 10-17$\mu$ |
| | Number of septa | | | 6-10 |
| | Length of conidiophores | | | 10-100$\mu$ |

La Rue suggested the discrepancy between measurements of spores derived from culture and those derived from lesions might be due to different culture conditions or a difference in strains. The difference between samples from Ceylon and from Sumatra he attributed to the fact that he had been measuring a random sample, whereas Petch may have been measuring only the larger spores.

The writer's measurements show a range which covers both those of Ceylon and Sumatra. Spores taken directly from the plant are always found to be larger on an average than those from culture. There is no doubt that the spores of *H.heveae* are very variable in size and that constituents of the culture medium affect their size (see page 51).

No evidence has been adduced for there being strains of *H.heveae* in Malaya, distinguishable by differences in spore size. Collections of the fungus from a number of localities have been grown in culture but no consistent differences found.

### Conidiophore

The conidiophore is extremely variable in size in culture. In typical fructifying spots on the leaf it averages 100 to 200$\mu$.

When well developed the conidiophore is geniculate in shape, as in many other Hyphomycetes. The spores are produced terminally; as they mature the axis grows on to produce further spores displacing the old spore laterally. This may be repeated several times. Microscopic examination of an actively growing culture usually shows three spores in position on the conidiophore. Below these three terminal spores will be the detachment scars of older spores which have been shed. The corresponding scar on the spore constitutes the hilum. The form of the hilum may be an important diagnostic feature in some species of *Helminthosporium*.

### Chlamydospores

Formation of chains of chlamydospores is well known in certain species of *Helminthosporium*, and is discussed as well as figured by Mitra* in a comparative study of several graminicolous species. Exactly similar chains are produced by old colonies of *H.heveae*, and by young colonies growing on any medium with a high sugar concentration. This gemmate mycelium replaces the normal mycelium entirely in colonies growing on saccharose-mannitol agar. The gemmae of older colonies tend to have thicker walls, and are thus more akin to true chlamydospores.

### Haustoria

These structures may be clearly seen in sections through young lesions, or in lesions that have been cleared for several days in lactic acid. They are spherical in shape, about 4$\mu$ in diameter, and arise from hyphae adpressed to the cell walls of the host. They bear a close resemblance to those figured for *Helminthosporium sacchari* by Butler (1918). When sugar cane was inoculated with spores of *Helminthosporium heveae* during the course of the search for a secondary host small brown spots were produced. Sectioning of these spots showed the presence of haustoria similar to those found in sections of the *Hevea* spot.

### White hyphal tufts

Ever since investigations were started, white tufts of mycelium have been observed on a wide variety of media. They were suspected of being abortive fruit bodies, but have never been found to develop spores. Similar tufts were observed by Mitra in his comparative studies mentioned above. As the development of these tufts slows down the rate of diametric increase of the colony, their formation was undesirable in experimental studies. Their appearance did not appear to be influenced by the type of medium, but did seem to be encouraged by any sudden temperature change. On transplanting the white tissue the normal dark mycelium is produced, so this cannot be considered a case of pleomorphism.

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* *Trans. Brit. Mycol. Soc.* 15 (1930-1) 254

FRUIT BODIES

No suggestion that *Helminthosporium heveae* possesses fruiting stages has hitherto been made. However, it is wellknown that certain species of *Helminthosporium* have perithecial stages, mainly in the genera *Leptosphaeria*, *Ophiobolus*, *Pyrenophora*, *Cochliobolus* and *Mycosphaerella*, all of which are classified in the *Sphaeriales*. Certain of these genera of Ascomycetes have pycnidial stages, for example *Mycosphaerella* has species of *Phyllosticta* and *Phoma*. It may be that *Helminthosporium heveae* has a pycnidial, as well as a perithecial, stage.

Several members of the *Sphaeriales* and the *Sphaeropsidales* have been encountered intimately associated with the *Helminthosporium* on disease lesions. Most of the fruit bodies examined have proved to be pycnidia, the genus *Septoria* being most frequently encountered. The most common perithecium falls into the genus *Mycosphaerella*. As yet no connection has been established between *Helminthosporium heveae* and these fungi.

## PHYSIOLOGY

*Helminthosporium heveae* is not an obligate parasite like *Oidium heveae* and *Dothidella ulei*, incapable of being grown on artificial media at all, or at least only on specially prepared media. It is a facultative parasite comparable with *Phytophthora*, capable of penetrating the tissue of its host and growing readily as a parasite, but also growing vigorously on artificial media. It is not a wound parasite as was earlier suggested by Petch (1905) who put forward the view that penetration might occur through insect punctures.

La Rue grew *H.heveae* successfully on *Hevea* decoction agar, following the method usually adopted with the growing of a parasite, by incorporating as much host tissue as possible into the medium. He found it grew well on bouillon agar too. La Rue also tested growth on raw potato and found a slight dry rot. He does not mention growth on inorganic media, and there does not appear to be any other work published on growth in culture.

La Rue's observations were confirmed in the present investigation, except that the initial growth of the mycelium did not always start off white and then darken on these media.

Variations in the medium have a considerable effect on spore production as well as on intensity and rate of growth. Although La Rue reported that sporing did not occur until the culture was quite old and the agar dry, it has been found that sporing occurs on quite young cultures and would often cease after 3 to 4 weeks.

INFLUENCE OF VARIOUS FACTORS ON GROWTH AND SPORULATION

INFLUENCE OF THE CULTURE MEDIUM—Fifteen media were tested at room temperature with results as summarised below.

Bouillon Agar. Growth woolly and slow. Sporing feeble.

Oat Meal Agar. The best medium for heavy spore production in the first few days of growth. Mycelial growth good but uneven.

Dox Agar. Growth good and even. As Dox agar is a homogeneous and wholly synthetic medium it is most suitable for experimental studies.

Rice Polishings Agar. Lies intermediate between starch agar and oat meal agar in its effect on both spore production and growth rate.

Corn Meal Agar. Good growth, but uneven owing to a very striking zonation effect not found on the other media. The zones consist of thin non-sporing bands (approximately 0.5 cm in width) alternating with dense sporing bands.

Starch Agar. Spore production commences early but is light. Mycelial growth very thin.

Potato Agar. A quite unsuitable medium bearing a slow, slimy growth.

Plain Agar. Supports a growth as rapid in diametric increase as the other media, but so thin that it is virtually invisible.

Potato Dextrose Agar. A fairly rapid growth tending to be woolly in later stages. Lacks the slimy nature of the pure potato medium.

Malt Agar. Growth slow and stunted. Not a suitable medium.

Hevea Decoction Agar. Good, but not exceptional, growth. Unsuitable for experimental studies owing to variation from stock to stock.

Phaseolus aureus Agar. Gives a thin growth comparable with starch agar, and zonation comparable with corn meal agar.

Prune Agar. Mycelium thin and feebly sporing. Margins of the colony lobed.

Brown's Agar. Mycelium spores well. Diametric increase slows down after a week and the edge of the colony tends to thicken.

Richards' Solution Agar. Mycelium spores thinly but consistently over the whole area; a few white mycelial clumps formed.

Richards' Solution. A very heavy grey growth on 30 cc of solution in a Roux bottle. No spore production.

Generalising from the above tests it appears that variation in the medium affects the intensity rather than the rate of growth. As would be expected heavy spore production slows down growth rate.

INFLUENCE OF CERTAIN NUTRIENTS—Variations of the ingredients within these standard media have been restricted to nutrients likely to exert their effect on the fungus within the leaf.

Mineral nutrients, including phosphorus and potassium, are unlikely to depart sufficiently from the normal, within a living leaf, to exert any marked effect on the fungus. But substances elaborated by the plant itself will make a big difference to the form and course of disease. Those elaborated in response to the parasite are considered later under 'Host Reaction'. Other

substances are those which provide the bulk of the food materials upon which both plant and fungus depend for their growth: carbohydrates and proteins with their degradation products. Chlorophyll and other pigments may contribute to the nutrition of the fungus.

Sugar concentration is known not only to affect growth of the colony, but also to affect spore size very markedly. In order to have some means of comparison with another species of *Helminthosporium* a method described by E. S. Elliot* was employed. A glucose-nitrate agar was taken as the basic medium, the glucose being varied from 0.0075% to 16%. Measurements were taken of the diameter of the colony after 10 days, and after a further 28 days in incubation. The mean of three plates for each concentration was recorded, and presented in Table II.

TABLE II

*The effect of sugar concentration on growth in culture*

| Percentage glucose in medium | Type of mycelial growth | Sporing | Diameter in cm after 10 days growth |
|---|---|---|---|
| 16 | Raised and woolly colonies | None | 2.8 |
| 8 | Thick and woolly | Light | 7.8 |
| 4 | „ „ „ | „ | 9.0 |
| 2 | „ „ „ | Medium | 9.0 |
| 1 | „ „ „ | „ | 9.0 |
| 0.5 | „ „ „ | „ | 9.0 |
| 0.25 | Medium growth, not woolly | Heavy | 8.0 |
| 0.125 | Thin growth, not woolly | Light | 8.0 |
| 0.06 | Very thin | Very light | 7.5 |
| 0.03 | „ „ | „ „ | 7.5 |
| 0.015 | Extremely thin | Extremely light | 4.0 |
| 0.0075 | No growth | None | 0 |

Spores formed on media of glucose concentrations above 2% tend to be malformed, whilst the mycelial growth produces numerous white tufts. Above 8% spore and mycelium production is not only abnormal, but is inhibited.

Measurements were made on spore length and cell number over the range 0.0075% - 2% and the measurements are shown in Table III.

The mean conidial length in the younger cultures appears to vary inversely with the amount of sugar, but to a much less marked degree than found for the species of *Helminthosporium* studied by Elliott. At higher concentrations this relationship no longer holds and spore shape and size become very variable. At 16% spore production ceases altogether. Only with considerably greater differences in length would cell numbers be expected to be consistently larger in longer spores. In this

* *Phytopathology* 39 (1949) 953

TABLE III

*The effect of sugar concentration on spore measurements*

| Glucose concentration in medium % | After 10 days | | After a further 28 days | |
|---|---|---|---|---|
| | Mean spore length in $\mu$ | Mean cell number | Mean spore length in $\mu$ | Mean cell number |
| 2 | 82.28 | 9.04 | 76.88 | 8.40 |
| 1 | 82.00 | 9.00 | — | — |
| 0.5 | 81.6 | 8.88 | 76.20 | 8.68 |
| 0.25 | 81.00 | 9.24 | 73.80 | 8.20 |
| 0.125 | 83.32 | 9.26 | 74.16 | 9.08 |
| 0.06 | 84.60 | 9.60 | 82.96 | 9.32 |
| 0.3 | 84.92 | 9.52 | 74.36 | 9.24 |
| 0.015 | 92.64 | 8.76 | 82.88 | 9.52 |
| 0.0075 | — no growth — | | — no growth — | |

narrower range the counts show that there is an average of 9 cells for all lengths.

It is seen that 0.25% represents a critical concentration above which there is an increase in growth density and a decrease in average spore length.

It is noteworthy that the greatest spore length recorded in these experiments (110$\mu$) is considerably less than the average for spores produced on the lesion.

Media in which either peptone, tyrosine or aspartic acid were incorporated, tended to favour woolly growth at the expense of spore production. Observations on quantitative variations have not been carried out.

INFLUENCE OF HYDROGEN ION CONCENTRATION—Czapek (Dox) agar was taken as the basic medium, and the pH varied with phosphate buffers.

TABLE IV

*Growth after 10 days at various pH values*

| pH | Consistency of agar | Growth | Sporing |
|---|---|---|---|
| 3.2 | liquid | none | — |
| 4.2 | very soft | normal | Heavy sporing |
| 4.8—8.0 | hard | normal | Heavy to medium sporing |
| 8.8 | hard | slow | Medium sporing |
| 10.2 | semi-liquid | slight | No sporing |
| 11.1 | liquid | none | — |

The figures given in Table IV show that the complete range for normal growth is from pH 4 to pH 8, a far greater range than that likely to be encountered within the leaf. Sporing was less heavy on the alkaline media than on the acid.

INFLUENCE OF TEMPERATURE—All the observations discussed below have been carried out with the fungus growing on 20 cc quantities of Dox agar in a 9 cm petri dish, and recorded as the mean diametric increase of six colonies held at the temperature to be measured.

A temperature as low as 40°C was found to markedly depress growth, and there was a tendency for the colony to build up vertically rather than spread out horizontally.

Table V shows the effect of temperatures between room temperature and 40°C on growth of the colony. As temperatures within this range are likely to be encountered where rubber is grown in the field, the measurements are of some significance in showing that *Helminthosporium* is likely to be more virulent at lower temperatures.

TABLE V

*Inhibitory effect of temperatures up to 40° on the growth of H.heveae*

(Figures give colony diameter in cms on Dox agar)

| Day | 1 | 2 | 3 | 6 | 9 |
|---|---|---|---|---|---|
| 27°C * - | 0.9 | 2.4 | 3.9 | 7.3 | >9 |
| 34°C - | 0.3 | 1.3 | 3.0 | 4.1 | 5.6 |
| 36°C - | 0.25 | 1.0 | 1.6 | 2.0 | 2.7 |
| 38°C - | 0.25 | 0.35 | 0.4 | 0.5 | 0.5 |
| 40°C - | no increase in diameter | | | | |

* room temperature

As the stage of growth reached at the time of placing in the incubator was found to be significant, the sets of six plates were inserted 0, 1, 2 and 3 days after inoculation. It will be noted from Table VI that the longer the colony has been growing at room temperature, the less its growth rate is depressed on exposure to the higher temperature. In all cases after removal from the higher temperature the edge of the colony became extremely irregular.

TABLE VI

*Reduction of inhibitory effect of temperature by growth under laboratory conditions prior to incubation*

(Figures give colony diameter in cms on Dox agar)

| Day | 1 | 2 | 3 | 6 | 9 |
|---|---|---|---|---|---|
| Three days prior to incubation | — | — | — | 7.0 | 8.2 |
| Two „ „ „ „ | — | — | 3.7 | 5.25 | 5.7 |
| One day „ „ „ | — | 2.25 | 2.5 | 3.0 | 3.3 |
| Incubated throughout at 38° | 0.25 | 0.35 | 0.4 | 0.5 | 0.5 |

It has not been possible to obtain figures for growth at temperatures below room temperature (taken as 27°C). *Helminthosporium heveae* will live and grow at temperatures as low as 5°C; the growth is, however, thin and tenuous.

INFLUENCE OF LIGHT—Light has very little effect on the rate of growth in culture, but it does affect sporulation, and accentuates the zonation obtained on certain media (Plate III). This zonation is formed by alternate sporing and non-sporing bands.

INFLUENCE OF MOISTURE—The fungus grows well in liquid media. It also grows well on dry twigs or rice stalks, provided a high humidity is maintained with the latter.

On agar media which are drying out there tends to be an increase in sporing and decrease in mycelium production.

## Influence of various factors on spore germination

Water is the first essential for germination to take place. The *Helminthosporium* spore is readily wetted, and even as small a quantity of moisture as that found on the damp surface of an apparently dry leaf will enable it to produce a germ tube. Germination readily takes place in distilled water and there is no need to postulate the formation of 'infection droplets' containing small quantities of carbonates and other substances secreted by the host, in order to account for appressorium formation and penetration.

The rapid growth of fresh spores in distilled water on a glass slide has been recorded by photomicrographs (Plate 4). A set taken over a period of 8 hours shows incipient germination within the first hour; a germ tube as long as the spore within four hours; and appressorium formation within eight hours.

Whilst dissolved substances may not be necessary to facilitate germination (germination of fresh spores in distilled water is 100%) certain dissolved substances may inhibit germination. This has been tested extensively and is discussed under Section VI, 'Control'.

The reaction of the liquid in which the spore is germinating is of some slight importance, but *Helminthosporium* is very tolerant of changes of pH over a wide range.

Spores were germinated, in a damp chamber, in drops of culture solution containing 5% mannitol with added phosphoric acid/potassium hydroxide to vary the pH. Observations made over a period of three days are summarised in Table VII, from which it is seen that conidia germinated over the pH range 3.0 to 11.5, and that optimum development of the hyphae occurred at pH 6.0.

TABLE VII

*Effect of pH on germinating conidia of Helminthosporium heveae*

| pH | Approximate germination % | Development of hyphae after 48 hours |
|---|---|---|
| 11.6 | 0 | |
| 11.5 | 95 | Length of germ tube no greater than the spore width |
| 10.7 | 95 | |
| 10.6 | 95 | Little branching, some chlamydospore formation |
| 9.4 | 95 | |
| 7.5 | 100 | Branching and a few appressoria |
| 6.4 | 100 | |
| 6.0 | 100 | Rich branching and appressorium formation |
| 5.0 | 100 | Branching and a few appressoria |
| 3.8 | 50 | No branching or chlamydospore formation |
| 3.3 | 10 | |
| 3.0 | 2 | |
| 2.9 | 0 | |

VIABILITY OF THE SPORE

Just as moisture is essential for the germination of the spore, so dryness is essential for prolonged retention of viability.

The period over which a quantity of air dried spores retain a high percentage viability when maintained at room temperature (taken as 27°C) is approximately 9 months.

At temperature higher than room temperature the life of the spore is shorter, until at temperatures of approximately 100°C it is measured in minutes. Some difference was found between the resistance to heat of spores on the leaf, or away from the leaf in direct contact with the glass on which they were supported. They proved less resistant in the latter case, and the figures quoted below are those for spores in contact with the glass.

At temperatures of 20°C, 30°C, 40°C, and 50°C, 100% germination was obtained after exposure of at least six days. At 60°C there was 93% germination after six days, and at 70°C there was a drop in germination after three days to 36%. At 80°C one day's exposure was lethal, at 90°C five hour's, and at 100°C at most one hour's exposure.

An attempt was made to find the thermal death point, that is the lowest temperature at which a brief exposure will kill all the spores. Mitra, during the work quoted on page 48, for several species of *Helminthosporium*, found this temperature to be just over 60°, determined by maintaining at the given temperature for 5 seconds. These observations were repeated for *H.heveae* using spores on a 12 mm cover glass at the bottom of a $\frac{1}{2}$ inch test tube into which a thermometer was inserted. The tube was enclosed in an oil jacket and heated. The source of heat was removed and the highest temperature reached recorded. The cover glass with spores was then removed, and after a drop of water had been added, placed in a damp chamber overnight.

It was found that with *H.heveae* temperature did not begin to exert any effect until over 80°C. Results were very erratic, and once there was 99.5% germination after heating to 135°C.

These figures are of interest in showing the extremely resistant nature of the *Helminthosporium spore.*

## IV. PATHOGENICITY

Under Section II, the disease has been considered independently of any knowledge of the biology of the organism responsible. It was noted, however, that the fungus *Helminthosporium heveae* always accompanied all stages of the disease. This fungus has been considered under Section III as an organism independent of any disease producing properties it may have.

It remains to consider the relationship between the fungus and the disease—the pathogenicity. The anatomical relationship has been studied by means of microscopic examination, and

the physiological relationship by inoculations under various conditions.

## TECHNIQUE

Hand sections have been cut and stained with lactophenol cotton blue for determining the position of the mycelium within the tissue. For information on the distribution of various types of tissue and whether normal or traumatic, chlor-zinc-iodide, phloroglucinol, ferric chloride, and Sudan III have been used.

Mechanically cut sections have been made with the Cambridge Rocking Microtome. Specimens have always been fixed in Flemming's weak solution, under vacuum, in the field.

A standard imbedding technique has been used throughout, after initial trials to find the most suitable process. Chloroform impregnation was chosen.

A wax of melting point 54.5°C has been found most suitable and sections have been cut to 8 $\mu$.

### Staining the fungus within the leaf

A variety of staining methods have been employed:

When only one stain was used Delafield's haematoxylin proved most satisfactory.

Using two stains, safranin O proved a satisfactory counter stain to the Delafield's haematoxylin. Better contrast resulted from using light green as a counterstain to safranin O. Stoughton's orange G and carbol thionin yielded very disappointing results, as the fungus failed completely to retain the purple stain, and uniform orange coloured sections resulted.

No double counterstaining was attempted. However the triple stain Pianese IIIB was used repeatedly but with very unsatisfactory results as again the fungus failed to retain the stain.

## PROOF OF PATHOGENICITY

It has been demonstrated that the fungus *Helminthosporium heveae* accompanies all cases of the disease, and that this fungus may be isolated from the diseased plant and grown in pure culture. Reproduction of the disease, by inoculation of healthy plants with spores from such a pure culture, and subsequent recovery of the same fungus, provide final proof as to the pathogen.

Repeated inoculations have been carried out for experimental purposes and periodical checks made by taking the pathogen back into culture. Although spores from culture readily reproduce the disease, spores taken directly from dried leaf tissue have usually been used in experimental work. Not only are the dry spores those responsible for infection in nature, but they are more readily obtained free from extraneous matter.

## SPORULATION

Characteristically a few large dark brown spores are found lying, together with prostrate conidiophores, on the surface of the semi-transparent centre of the typical 'Bird's Eye'. Although this represents a rather late stage in sporulation it is the form

described in the published descriptions, and a reliable diagnostic character. Sporulation begins on the typical lesion by a large number of conidiophores springing up from the dead, but still opaque, centre of a spot already delimited by the sharp brown margin. This becomes covered with a mass of large brown spores, and the mesophyll and palisade tissues become absorbed, leaving only the upper and lower cuticle which form the characteristic translucent spot.

Heavy sporulation usually occurs in periods which are probably related to weather, and particularly to the age of the plants. Plants growing on a patch of peaty soil with an exceptionally high water table bore heavily sporing lesions continuously for eight months, despite marked fluctuations of sporing in the surrounding plantings growing under drier conditions. It has been noted that the first lesions formed on a plant usually spore very heavily, but after some weeks sporing drops considerably. With plants that have escaped infection when very young, either owing to their growing in isolation or under temporary shade, sporulation takes the same course and is very heavy for the first few weeks, whatever the prevailing state of sporulation in the surrounding plantings.

Spores produced on small spots are fewer in number and smaller in size on the average than those produced by larger spots. If rampant infection has occurred a very large number of smallish spores cover the dead tissue entirely.

It is significant that whereas infection can take place through either the upper or lower surface of the leaf with equal facility the majority of the spores are produced on the lower surface only. Anatomically the two surfaces of the leaf differ very considerably. The cuticle on the upper surface is smooth except for a network of fine striae appearing as papillae in transverse section; underneath lies the epidermis, from which stomata are completely absent, and underneath this the palisade layer. The lower cuticle is produced into a dense reticulation of interlocking ridges, appearing as hairs in transverse section; underneath lies the epidermis, containing numerous stomata, and then mesophyll tissue.

To eliminate the possibility of insolation or gravitational influences being responsible for this difference in sporing, leaves at various stages were inverted, by twisting about the petiole, and held in position by means of a clamp consisting of a split bamboo attached horizontally to two bamboo uprights. Heavy sporing always occurred on the abaxial surface whichever surface was inoculated.

The stage of very heavy sporing is of characteristic appearance, even as seen from the upper surface. The lesions show as light brown patches on a leaf that is of a striking yellow green shade, thin in texture and often quite small. Usually all the lesions on all the leaves of a whorl pass through this stage simultaneously. It may occur on young seedlings bearing only their first pair of leaves. It appears to be commoner on very young plants and accounts for the very rapid initial spread of infection in a nursery.

Even at this heavy sporing stage, the number of spores is not very large, the fungus depending for its survival on a small number of large resistant spores rather than on a large number of small spores of which only a few would survive.

Suppose the average number of leaflets bearing sporing lesions on a young plant at maximum sporing to be 126 (taking the latest flush to reach maturity and the two previous flushes). On each leaflet may be 20 to 30 lesions, say 25. Each lesion may bear 150 spores, so spore production over one fortnight (the normal duration of heavy sporing) may be 126 x 25 x 150 = 472,500 spores per plant; not a large number compared, for example, with *Hemileia vastatrix* on coffee which has been recorded as producing 159,000 uredospores a day from one spot, and bearing 60 or 70 spots on each leaf.

Tests on the *Helminthosporium* spores *in vitro* have shown how resistant they are to heat, poisons, and prolonged storage. The food reserves of the comparatively large spore, and the resistant coat, both contribute to the production of a spore that is capable of withstanding most unfavourable conditions (viability in the first few months is 99—100%) and capable of very rapid penetration and growth once it has landed on the host leaf.

As described on page 48 the spores come to be born laterally on an erect conidiophore, having been produced terminally and then displaced by monopodial branching. The actual point of attachment consists of a slight swelling below a septum on the conidiophore. The spore is fixed to this by its hilum. It is very readily detached, but tends to cling to the leaf on which it is produced. Spores which appear in this manner as protrusions of the tip of the fertile hyphae are of the exogenous type, a type which is ordinarily disseminated by wind.

## DISSEMINATION

The large size and flattened shape of the *Helminthosporium* spore, its mode of detachment from the conidiophore, and the dry, as distinct from slimy, sporulation, all point to dissemination by wind. Observations on the many well known *Helminthosporium* diseases of cereals indicate wind dissemination to be a characteristic of the genus.

La Rue (1923) suggested wind dissemination for *H.heveae* after he had demonstrated that systemic infection of the shoots did not take place.

During the course of the present studies, tests were carried out in the experimental plots at Sungei Buloh, and it was found that enclosing flushes in necked bell jars would protect the plant from infection. In this method the jars were suspended from a wooden tripod, neck downwards over a terminal bud. The neck was blocked round the stem with cotton wool, and the flanged base (uppermost) sealed with a glass plate smeared with rubber grease. Subsequent inoculations were carried out by removing the glass plate, thus enabling one to demonstrate conditions inside the bell jar to be favourable for disease development.

Later, bags of 'Alkathene' were used instead of the heavy glass jars. Enclosure in an 'Alkathene' bag ensured complete protection from disease.

To demonstrate the presence of spores in the air of the nurseries spore traps were designed:

The spore-trap used in 1948 consisted of an aluminium box 18 in x 18 in x 9 in with an opening cut at one corner and a tail fixed to the diagonally opposite corner. The box was pivoted to swing freely, horizontally, on the top of a five foot iron pillar. At the mouth, and at several positions inside the box, were placed pairs of 3 in x 1 in slides. The slides were coated with glycerol, gelatine, vaseline, or agar. Agar was found to be the most satisfactory. The trap was too heavy to respond to light air currents, and readily flooded in rain. Under favourable conditions of dry windy weather catches were very low—only 2 or 3 spores per 3 in x 1 in slide in 24 hours. Various positions for the slides were tried: vertical and horizontal, near the mouth and near the back. As this did not improve the spore yield, alternative models were designed.

The type of trap tested in 1949 consisted of a light metal shaft 2 feet long, pivoted in the middle, and free to swing horizontally, at one end was fixed an aluminium tail, at the other a spring metal clip holding a 7.5 cm half petri dish. The clip was positioned so that the face of the dish was at 45° to the ground. Agar was found to be an ideal retentive medium, and occasionally malt agar was used, as this allowed unknown spores to germinate and grow to a recognisable form.

This trap was far more sensitive to slight wind change than the box type, its inverted surface prevented flooding during rain. and its simple construction enabled duplicates to be set up for comparing spore populations in different places at the same time. However the yield of spores was disappointingly small, being four or five spores per dish surface in 24 hours. So small a yield made any comparison between sun and shade and low or high position quite valueless.

In 1950 a different form of trap proved very satisfactory for demonstrating the presence of spores in the air. It was similar in construction to a whirling hygrometer and consisted of two laths 9 inches long and $1\frac{1}{2}$ inches wide positioned parallel to each other, face to face $1\frac{1}{4}$ inches apart. At one end was a spindle and handle, at the other end slots into which fitted a pair of 3 in x 1 in slides back to back, half an inch apart, and at right angles to the laths. It was found best to insert one uncoated slide for balance, and the second, coated, slide so placed that the surface struck the air when rotated in a clockwise direction. A two minute exposure was given and the results expressed as spores per square half inch.

Specimen results are as shown in Table VIII.

Results as given by sticky spore traps provide little more information than that there are spores present in the air at a certain place and time. What proportion of the spores in the air are actually caught, how rapidly the airborne spores are deposited, and how far they may drift in air currents of various

strangths depend on knowledge obtainable only from laboratory tests with wind tunnels, settling towers, and spore dispersal chambers, in which there is a known number of spores per unit volume.

TABLE VIII

*Numbers of spores per square half inch caught by whirling trap*

| Conditions in nursery | 2 min exposure | 5 min exposure |
|---|---|---|
| Air still in lightly sporing nursery | – | – |
| Air moving in lightly sporing nursery | – | – |
| Striking lightly sporing plants with trap | 3 | 5 |
| Air still in heavily sporing nursery | – | – |
| Air moving in heavily sporing nursery | 1 | 2 |
| Striking heavily sporing plants with trap | 6 | 17 |

An attempt was made to estimate rate of fall by observing the settling of a spore cloud in a brightly lighted tower. The tower consisted of a vertical glass tube 1 metre in length and 3 cm in diameter clamped above a 6v 30w microscope lamp. Dry spores deposited on a coverslip were scraped off with a needle, and the time of fall between two points 30 cm apart taken with a stop watch. The time of fall averaged 16-17 seconds, giving a rate of fall of approximately 2 cm per second. This compares with figures of approximately 0.05 cm/sec published for spores of *Lycoperdon*, and 1 cm/sec for the majority of fungus spores.

The spores of *Helminthosporium* appear to have a slightly adherent coat which helps them to remain on the new host leaf, but which retards liberation from the old host. The spores leave the point of attachment to the conidiophore readily enough, but tend to cling to the lesion. The glistening spore coat is not water repellant and the spores are readily wetted. After rain or dew on a sporing leaf the spores may be found in the drops of water. The water distributes them over the leaf surface, on which they may give rise to secondary infection or may dry out and be blown away. A warm windy day would be ideal for dissemination.

It is noticed that leaves of a flush in the neighbourhood of a freely sporing lesion rapidly become infected during the time that they remain in a susceptible state. Such local dissemination could be accomplished either by the leaves blowing together, by insects walking over the leaves, or by wind itself.

An excellent opportunity for the study of the spread from plant to plant within a large area was provided by a 50 acre extent of seedlings at Sungei Buloh. This showed that dissemination follows a similar plan as in the small area. The progression of infection could be followed steadily as the disease spread from the boundary, where it first appeared, towards the centre. From this and other observations it is clear that the majority of spores are carried only a few yards by the wind. The spread through a neighbouring field planting was observed

to be slightly slower than through the nursery planting. Far ahead of the main line of advance isolated diseased plants would be found, and this sudden appearance of an odd infected plant amongst healthy plants was characteristic of the spread of the disease through the Sungei Buloh nursery. It contrasts with the 'blasting' type of windborne infection found, for example, with the aecidiospores of *Puccinia graminis*.

Isolated infections produced by an odd spore carried a long distance could be responsible for spreading the disease from one nursery to another within the same locality. La Rue (1922) suggested such infection originated from mature trees, but it is not necessary to postulate infection from this source; moreover, in Malaya at least, lesions on the leaves of mature trees have been encountered only very rarely, and then not freely sporing. In view of the restricted rate of spread there may be considerable scope for isolating nurseries, and by the time infection has been able to build up the seedlings will have become sufficiently well grown to be ready for budding or transplanting. This occurred in an isolated nursery under continuous observation, and hand picking of such lesions as did appear kept down infection still longer.

Spread from one nursery to another some distance away is more likely to be due to human agency in carrying and planting of diseased plants, than due to windborne spores.

Spread from country to country is also likely to occur through the fungus being carried on planting material. Without further knowledge of its distribution throughout the world and the date of its first appearance in various countries it is not possible to suggest its country of origin.

There is, of course, the possibility that the fungus was already widespread on some other host, but none has yet been encountered.

## INFECTION

This section, together with the subsequent section 'Host Reaction', covers the subject 'host-parasite relations'. The information has been obtained by examination of artificially inoculated plants growing in the open, and has led to observations on the course of the disease on naturally infected plants in the field.

### Inoculation methods

Although many observations made in the field are referred to in the literature no example of a successful inoculation has been recorded.

In the present studies the usual inoculation method of spraying spore suspensions by means of an atomiser was tried at first, but proved a failure. Spores would germinate in the drops of moisture, but failed to penetrate the leaf.

Nor was inoculation successful (either with or without wounding) under cover slips fastened to the leaf with vaseline.

Up to the end of 1948 a method of inoculation with a single spore, so as to observe the subsequent process of spot formation, had not been devised. Typical spots had been obtained

on potted plants in the open, however, by placing drops of spore-agar paste taken from the fungus growing in culture on to leaves 2 cm long.

At the beginning of 1949 a satisfactory method of laboratory inoculation was developed, capable of rendering the process of spot formation from a single spore readily observable. Diseased leaves bearing freely sporulating lesions were dried in the air and the spores scraped off directly onto the leaf to be infected. The plant was then enclosed in a bell jar together with a beaker of water. Spots developed within 24 hours. The spores of *Helminthosporium* proved sufficiently large to be positioned on the leaf with a fine needle.

At the end of 1949 the complete absence of disease from a small nursery on the Institute's premises provided an excellent opportunity for carrying out artificial inoculations under field conditions. The procedure was exactly the same as that employed on potted plants, by 'spattering' dry spores on to the leaf surface. No provision for maintaining a high humidity was found to be necessary in order to secure infection and typical lesions. Results of inoculations carried out in this way are shown in Plates 5, 6 and 7.

### Conditions influencing infection

Conditions which influence the parasite before it reaches its host have been discussed in the section 'Dissemination', factors influencing germination of the spore are discussed under 'Physiology', and factors concerning receptivity of the host under 'Host Reaction'.

When all three conditions, access, germination and receptivity, are fulfilled infection can take place. It is largely through these conditions that the environment exerts its effect.

### Stage before penetration

The spore, having reached the leaf surface, is at the critical stage in its life history. It is a free-living organism with its subsequent life depending on three factors: conditions prevailing favourable for its germination, sufficient nourishment to grow once germinated, and successful attachment to the susceptible leaf. The cuticle that covers the upper and lower epidermis of the rubber leaf is not smooth but finely furrowed. On the upper surface these furrows take the form of fine striations, appearing as papillae in cross section. On the lower surface the furrows are deep and the raised tissue ridge like, appearing like hairs in cross section. Newly landed spores are most abundant on the upper surface. One would expect the spore which alights on the lower surface to be favoured by shelter, a more easily gripped cuticle, and probably a higher surface humidity. Inoculations show upper and lower surfaces to be infected with equal ease.

To the finely furrowed surface of the cuticle the *Helminthosporium* spore, which itself has a glistening coating, readily adheres. Adhesion is particularly close to a young leaf with a soft cuticle, it being difficult to pick the spore off such a leaf with a needle and quite impossible to shake it off.

Dew covers the leaf with fine droplets, whereas rain deposits only a few large drops. The spore germinates readily in water and the germ tube tends to grow up into the water drop and branch. The spore is therefore much less likely to penetrate a leaf on which water is standing, despite the high humidity and rapid growth under these conditions.

The spore is large, and capable of supplying sufficient nourishment to support abundant mycelial growth in distilled water. Hence the germ tube is readily produced and maintained during appressorium formation and penetration. Appressoria readily form on clean glass slides so the stimulus is most likely one sided and mechanical. The grip of a large appressorium in intimate contact with the striated cuticle is very tenacious. It is not possible to wash the spore off, even with a strong jet of water.

The appressoria are readily seen in surface view on the leaf by staining cut out spots with lactophenol-cotton blue.

### Phase of penetration

There are four ways in which leaves may be penetrated: directly through the epidermis, through hydathodes, through the stomata, or through wounds.

The earliest worker, Petch, suggested wounds caused by insect puncture provided the means of entry. Although *H.heveae* can certainly grow on wounded tissue, it readily penetrates unwounded leaves and this is observed to be its usual mode of infection.

Stomata and hydathodes are very numerous on the lower side of the rubber leaf, and it is to be expected that *H.heveae* could readily infect through them. But penetration readily occurs on the upper side of the leaf where there are few hydathodes and no stomata. It can also take place anywhere on the lower surface of the leaf. Sections have been obtained through the appressorium and leaf, and although the fine processess illustrated for other leaf fungi in the literature have not been demonstrated they are most probably formed.

## HOST REACTION

When the penetrating hypha has pierced the cuticle it comes into contact with the living host cells and induces a reaction which varies greatly according to their physiological state.

The first symptom of this reaction is a sinking of the cuticle at the point of penetration, and the appearance of a yellow halo round it. This takes place within 12 hours. The subsequent course of the infection depends entirely on the condition of the host. There are different types of development on sun and shade, young and old, juvenile and adult leaves, although borne on plants growing under similar soil and climatic conditions. There is a range of different types of infection on stages of development ranging from the small brown, through the flaccid green, to the fully formed leaf. This is illustrated by the results of a series of inoculations carried out under normal field conditions shown in Table IX. Other experimental inoculations carried out to test the effect of alteration of environmental factors, are reported on page 71.

TABLE IX

*Different types of infection resulting from inoculation with H.heveae of leaves at various stages of development*

| State of leaf at inoculation | Approx age in days | Form of lesion after 3 days | Distortion of leaf | No. of days to sporing |
|---|---|---|---|---|
| Emerging from bud | 0 | rampant infection | severe | 5 |
| Three leaflets distinguishable | 2 | as above | severe | 5 |
| Lamina folded | 4 | large spreading lesions | severe | 5 |
| Newly opened lamina | 6 | as above | severe | 4 |
| Small, flat, shining pigmented | 8 | well defined spots diameter 0.5 mm | severe | 4 |
| Medium, shining turgid | 9 | as above | moderate | 4 |
| Large, shining turgid | 10 | as above | moderate | 4 |
| Shining, dull at base | 13 | very well defined diameter 1 mm | slight | 5 |
| Dull brown pigment | 14 | large well defined diameter 1 mm | severe | 5 |
| Trace of brown pigment present | 15 | average in size well defined | slight | 6 |
| Large, vertical, cuticle soft | 16 | large, with dead centre | none | 6 |
| Large, vertical, cuticle just hardening | 17 | typical, with a thin margin | none | 6 |
| On point of rising | 19 | typical, with thick margins | none | 6 |
| Leaflet at 45° | 24 | not susceptible | | |
| Newly horizontal | 29 | not susceptible | | |

Table IX shows the importance of the distribution of the anthocyanin pigment as an indicator of changes in cuticle hardness and host reaction. It is the final hardening of the cuticle which occurs at the time of raising of the lamina that prevents penetration of the spore.

It was found that when this newly hardened cuticle was damaged by abrasion with fine emery cloth, sufficiently shallowly not to rupture the latex vessels, a subsequent inoculation with *Helminthosporium* spores would take successfully. Deeper scratching with a fine needle resulted in the formation of the areas of dead tissue, on which *Helminthosporium* would grow, but then clearly as a saprophyte.

The critical stage of cuticle thickness may be determined by gripping the petiole with one hand and drawing the thumb and forefinger of the other down the leaf. A leaf strong enough to produce a sound is already past the susceptible stage, whereas a soft leaf, which would merely tear, will still be susceptible. Inoculations were made on leaves just past the stage of susceptibility, and the spores stained and examined. Most of the spores had germinated, but after three days were shrivelled and the hyphae thick, branched, and with large appressoria. On a susceptible leaf the germ tube normally grows to a length of about 50 $\mu$ then forms an appressorium and penetrates.

A useful comparison may be made between *Helminthosporium heveae* and another facultative parasite *Botrytis cinerea*, the pathogenicity of which has been extensively studied in temperate regions. For instance the yellow halo round the point of penetration may be compared with that produced by *Botrytis cinerea* after penetration of the cuticle. An attempt was made to prepare an extract of the germ tube of *H.heveae* as was done by Brown* with *Botrytis*, but no disorganisation when applied to needle punctures on the leaves was obtained.

A striking feature of the *Helminthosporium* infection is the rapid diffusion from the point of penetration of the disorganising enzyme present in the invading hypha. The rate of this diffusion is marked by a pronounced reaction in the host cells and the production of a thin line of black pigment. In one inoculated plant this black line was laid down within two days, forming a circle of radius 1-2 mm with the point of penetration as centre. Within the next two days the circumscribed leaf tissue had died, and after yet another two days, *i.e.* on the sixth day, this dead tissue was white in colour and bearing conidiophores. The nature of the black delimiting pigment is of considerable interest, as it is the basis of the dark brown margin which marks the rim of the 'bird's eye'. Microchemical tests have demonstrated the presence of deposits of phenolic bodies. There appeard to be no lignification or suberisation, although vascular elements passing through the margin did stain with phloroglucinol. However the primary delimitation does not appear to be a tannin, but a melanin deposition. Melanin formation is commonly found in traumatic tissue in plants with a tyrosin/tyrosinase system.

In *Vicia faba* for example wounding or invasion by *Botrytis* results in disorganisation of the cells and oxidation of tyrosin, through pink and red intermediaries, to melanin†. The rubber leaf does not give the brown reaction on wounding which characterises the catechol oxidase class of plants, and tests with p-cresol on a water extract of leaf tissue that had been macerated under 95% alcohol indicated tyrosinase to be absent. Tyrosin itself, however, may be present and may possibly react with tyrosinase, or some similar oxidative system, produced by the invading fungus. The abortive spots produced by *H.heveae* on leaves older than the optimum for infection, or on shade leaves, very often have a reddish or reddish brown colour.

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* Brown, W. *Ann. Bot., Lond.* **29** (1915) 313

† Wilson, A.R. *Ann. Appl. Biol.* 24 (1937) 258

The limitation in size of the normal lesion, for any given age at infection, may be due to inhibition by the fungus' own staling products until growth is so feeble that a response on the part of the host may set in. Alternatively one may assume that at the commencement of infection a toxin derived from the germ tube is present, which rapidly kills the host cells. As the circumference of the lesion increases, the area of the leaf itself (all the living cells of which are potential producers of 'anti-toxin') is reduced by a negligible extent. But the area within the margin into which the fungus toxin is being secreted increases markedly. Finally comes a toxin/anti-toxin equilibrium which results in the laying down of a barrier. Then follows deposition of tannins resulting in a typical spot.

As seen in Table VI inoculation of very young leaves results in 'rampant infection', an effect frequently observed in the field when there is a high incidence of disease. At this early stage the host leaf lacks the mechanism for laying down a delimiting barrier round the fungus, and local resistance therefore breaks down. The result is severe distortion of the lamina and sometimes defoliation. The fungus grows vigorously and spores copiously over the whole of the surface. In severe cases repeated defoliation results in a swelling of the upper part of the main shoot, producing the 'sausage top' effect (Plate 1). Even the rampant infection may be limited to the leaf tip, when the limiting effect is perhaps due to drying out of the leaf in advance of the parasitic hyphae, so that a dead zone is formed right across the leaf bordered by leaf tissue already past susceptibility.

Two types of infection, comparable with the above, were recognized by Wilson in Chocolate Spot of *Vicia faba* and named 'aggressive' and 'non-aggressive'. The 'aggressive' corresponds with the 'rampant' infection of *Helminthosporium*, and the 'non-aggressive' with the discrete 'Bird's Eye' lesions.

Within the host, *Helminthosporium* does not become systemic and is limited to chlorophyll containing tissues. By external infection the fungus may penetrate chlorophyllous tissue of the petiole or young stem, producing elongated black streaks. These stems or petiole lesions do not spore freely, and it is only on the leaf that the typical lesions occur. The fungus does not disintegrate the lignified, suberised or cuticularised tissues of the leaf, so that a spot on which *H.heveae* has finished developing shows as a translucent area consisting of the upper and lower cuticles, and crossed by veins of vascular tissue. Often secondary fungi invade this translucent area, particularly members of the Sphaeriaceae and the Sphaerioidaceae. Considerable attention has had to be paid to the perithecia and pycnidia which are the fruit bodies of these families because of the possibility of their being stages of *Helminthosporium*, but no connection with this fungus has been established (page 49).

### Defensive reaction

The preceding description of the anatomical and physiological changes which follow penetration may be considered from the point of view of symptoms of the diseases *i.e.* as reactions that

are peculiarly against *H.heveae*, or as defensive reactions which lead to checking of disease and consequent survival of the plant, despite retardation of growth.

'Defensive reaction' leads to the important consideration of disease resistance.

## DISEASE RESISTANCE

Apart from absence of the disease due to environmental factors (*i.e.* 'disease escape') there is a considerable variation in resistance to the disease among plants of different ages and different species of *Hevea*.

Observation on *Hevea spruceana* x *Hevea brasiliensis* hybrids showed them to be as susceptible to the disease as pure *H.brasiliensis*. A plant of pure *Hevea spruceana* has been seen heavily attacked. A number of young plants of *Hevea benthamiana* exposed to infection developed only a few small spots and appeared on superficial examination to be completely free from disease. The young leaves could be readily infected by artificial inoculation but the typical large spot with clear central area never developed.

Mature trees have been found to be resistant to the disease; large numbers of leaves have been examined from a variety of sources but only very occasionally were lesions of *Helminthosporium* encountered. Even then the spots were small, with only one or two spores. The resistance extends down to an age of about three years. It might be expected that this resistance to the disease (which includes even the most juvenile leaves of mature plants) would extend to the leaves of buddings. Physiologically the shoots of a clone such as Avros 50, which was established from a mature tree in 1919, are now approaching 50 years old. On several occasions groups of buddings of various clones have been virtually free from *Helminthosporium* although growing amongst beds of seedlings that have been heavily attacked. Occasionally a budded (clonal) shoot has been observed virtually free from the disease whilst a stock (seedling) shoot, growing from the same root system, has been heavily attacked (Plate 8). The delicate juvenile leaves of the buddings would appear to be anatomically as susceptible to fungus attack as those of seedlings. However immunity of budded material is by no means general, and very often buddings and seedlings are attacked to a similar extent. When the effect of immunity from *Helminthosporium* does occur it is very often masked from superficial examination by an attack of *Gloeosporium alborubrum*, a fungus to which buddings appear to be rather more susceptible than seedlings. No evidence was found that freedom from Bird's Eye Spot was confined to any particular clone. The leaves of Tj.1 have a rather leathery texture, but when these leaves are forming they may or may not be attacked. Clone L.C.B.870, from Ceylon, is reputed to be resistant to *Oidium* attack; when planted amongst seedlings in a nursery heavily attacked by *Helminthosporium* it was found to be as susceptible as the seedlings themselves.

An obvious approach is to seek resistant plants amongst the great variety of genetic material provided in field nurseries.

J. R. Weir (1929*b*) remarks that he occasionally observed individual seedlings in nurseries to exhibit a high degree of resistance to *Helminthosporium*. Repeated search of the 200 acre nursery area in Sungei Buloh has failed to provide any plants free from the disease. Within any given locality all plants were infected to a remarkably consistent degree. Some plants that appeared to be slightly less heavily infected were selected and transplanted to an area exposed to infection. They rapidly became as badly spotted as the surrounding plants.

# V. ENVIRONMENT

So far consideration has been given to factors operating upon the fungus, the host, and on the host artificially inoculated with the fungus. All have been considered from the point of view of the individual plant. It remains to consider these same factors operating on a field scale, and apply knowledge gained in the laboratory to the same factors operating in nature, where host and pathogen occur together and produce the disease.

Essentially study of the environment is the study of the ecology of the rubber plant, and the factors which render it diseased. To a lesser extent it is a study of the ecology of the fungus and the factors in nature which enable it to produce disease. Maintenance of conditions favourable to the growth of the host will govern the conditions under which the fungus has to grow, hence study of the environment in relation to the fungus is concerned with small variations in factors already largely defined.

Climatic factors comprise rainfall, illumination, temperature and air movement.

Soil factors comprise soil structure, drainage, and nutrient content.

Biological factors consist of the part played by plants (in harbouring disease), animals (in spreading disease) and above all man as a cultivator and distributing agent.

## CLIMATIC FACTORS IN THE ENVIRONMENT

### Rainfall

Conflicting reports occur in the literature concerning the influence of rainfall. Barat (1930) states that the disease caused defoliation of young trees at the onset of the rainy season. In 1931 he states that a violent outbreak was occasioned by the cold December rains. Beeley (1936) mentions it as giving considerable trouble during the dry weather in February. Kevorkian (1948) reports an outbreak following on a three week drought. Leefmans (1927) reports defoliation during the west monsoon. Martin (1947) reports it as prevalent in Mexico during the dry season. Sanderson (1931) says 'the long period of dry weather was no doubt a most important contributory factor'. Venkataramani (1949*b*) suggests a severe drought

may have been responsible for the repeated defoliation he observed during the 1947-8 season.

Weekly observations on disease incidence, sporing, and plant growth have been made over a period of 18 months at several points in the Sungei Buloh nursery. It has been found that there is by no means a clear correlation between rainfall and disease. There has been no question of 'outbreak', for in both the 1949 and the 1950 plantings the disease started on the first planted seedlings and steadily increased in severity. The drought during the 1949 planting season, however, retarded the plants severely, and the effect of this was evident throughout the year. In 1950 there was no drought and growth from the beginning of the year was more vigorous. Although the disease was very widespread and the leaves were bearing many lesions, it could not be called severe during 1950. 1951 opened with a period of high rainfall and, although severity was variable, there were places in which infection was very slight.

The fungus itself seems not to be dependent on wet conditions for spread or infection, and can develop disease with equal facility in both wet and dry weather. It would appear that it is the plant which is influenced by the weather, wet conditions favouring vigorous growth and a consequent reduction in the importance of the disease.

### Light and shade

It might be expected that the seedlings of a forest tree such as *Hevea brasiliensis* would be adapted to growth under shade, rather than open conditions. It had been noticed for some years that rubber seedlings growing in the shade were relatively free from disease, despite suffering from retardation due to root competition with the mature trees. The first published experiments on growing rubber under artificial shade were those carried out by van Baalen in Java*. Van Baalen's shade of wood and lalang was successful in reducing the incidence of diseases and pests, and suppressed weed growth. Bird's Eye Spot is not specifically mentioned however.

Murray (1939), from Ceylon, reports that light overhead shade consisting of palm frond thatch ('cadjan') at a height of 7 feet, gave a substantial degree of control of Bird's Eye Spot.

In 1949 one half acre of planted nursery at the R.R.I. Experiment Station was covered by a shade measuring 120′ x 180′. It consisted of palm frond thatch ('atap') supported on a wooden frame 7 feet above the ground, as shown in Plate 10. In 1950 a similar, but less dense, shade was erected one quarter of an acre in extent. Both the half and the quarter acre shade were completely successful in eliminating the disease. However the plants within were found to be small in both girth and height compared with those outside. At the edge there was a distinct marginal effect, taller (but diseased) plants extending to approximately 9 feet within the shade. The most pronounced illustration of the contrast between sun and shade conditions was provided by plants that had grown to a height exceeding 7 feet and had consequently pierced the atap. Plate

* *De Bergcultures* 12 (1938) 664

11 shows the difference between the ultimate (sun) flush and the penultimate (shade) flush.

A shade of this type, if it were to be used in estate practice, would prove very expensive. Certain smaller scale trials have been carried out with other materials, which might be used year after year. Wire netting interwoven within lalang straw might be employed, supported on a framework of thick bamboos. A strip of hessian cloth four feet in width was found to give some degree of protection to plants underneath, and it might be possible to stretch the cloth season after season over a permanent frame.

However, the ideal shade would be a living tree, for convenience rubber. The shade of mature rubber is too heavy for vigorous growth of the seedlings, but trials are being made planting small nurseries under a thinner rubber shade and isolating them by cutting the roots of the mature trees.

A planting of *Gliricidia sepium* has been made to observe its suitability for use as a shade tree in nurseries.

The effect of the shade is, of course, by no means only to reduce the light intensity. It will act as a protective screen slowing down environmental changes in rainfall, humidity, wind currents, temperature, and evaporation. It will have the effect of smoothing out small fluctuations in these factors, so producing a much more even environment. Rainfall would be expected to exert its effect, but not immediately, as it must penetrate the atap or drain through the soil before reaching the plants. Humidity might be expected to be higher and temperature lower than in a comparable area in the open. This was investigated by placing recording instruments in Stevenson screens; one outside and one inside the shade. The differences between within and without were not marked. The temperature of the sun leaves will rise well above the shade figures when exposed to direct insolation, and the possible influence of these factors upon the course of the disease is discussed on page 73.

There is the absolute effect of overhead cover in permanently reducing light intensity, and in preventing deposition of dew. It is to these two latter effects that attention must be paid. In general it is known that, with plants, light intensity is of the greatest importance in influencing growth, but dew deposition of little or no importance. With parasitic fungi it is known that light has a considerable effect on both growth and orientation. Dew, as well, is a vital factor in providing the moisture essential for germination and penetration of a large number of species of parasitic fungi.

EFFECT OF SHADE ON THE PLANT—Sun and shade leaves in general have long been recognised as showing certain biological differences. Shade leaves tend to be thinner, with a greater volume of intercellular space, a greater ratio of green to yellow pigments, and a smaller number of stomata. The rubber leaf has been found to be quite typical in these respects. Stomatal counts gave an average of 410 per sq mm on a sun leaf, compared with 219 per sq mm on a shade leaf of similar age.

Chemically the sun and shade leaves show several differences, and these are likely to be of importance in relation to the nutrition of a parasite. An important difference is that in nitrogen content; one typical sun sample contained 4.40% (calculated on dry matter) compared with 5.10% for shade leaves produced by plants of similar age growing in a similar situation. The tannin content of sun leaves has been found to be slightly higher and to differ qualitatively from that of shade leaves. Extracts from sun leaves usually give a gallo-tannin reaction and those from shade leaves catechol-tannin. Attempts to link this with a different degree of toxicity to the fungus by growing colonies on different media containing tannin have so far yielded inconclusive results.

Taking the rubber plant as a whole, shade has a deleterious effect on growth, and shade plants are lacking in girth and height compared with sun plants of similar age growing in a similar situation.

EFFECT OF DEW ON THE PLANT—Dew may be an important source of water for certain epiphytes and xerophytes, and to some extent will interfere with transpiration. For a plant growing in a climate with abundant rainfall it is not likely to have any important effect on growth.

EFFECT OF SHADE ON THE FUNGUS—It has been shown from laboratory studies that cultural form is affected by light or shade, notably the zonation closely connected with sporing.

Small scale experiments carried out with potted plants showed light to be of considerable importance in its influence on infection (Table X). The plants were covered with bell jars to ensure a constant 100% humidity and optimum conditions for infection throughout. The light intensity was measured by a Weston 'Master' exposure meter.

TABLE X

*Effect of light on infection by Helminthosporium heveae. Seedlings 1 month old, at 100% relative humidity, artificially inoculated*

| Light conditions | Meter reading | Number of lesions | Condition after 5 days |
|---|---|---|---|
| Full sunlight | 200 | many | Lesions sporing heavily<br>Severe distortion of leaves |
| Light shade | 75 | many | Lesions sporing heavily |
| Heavy shade | 30 | many | Each lesion producing 1-2 spores |
| Indoors | 0.4 | few | Typical, but small, lesions |
| Shade indoors | 0.2 | few | Small lesions |
| Darkness | 0 | less than two per leaflet | Many germinated spores failed to penetrate |

Table X shows that germination of the spores occurs readily on the leaf surface whether in the light or dark. Under dark conditions, however, they appear to be incapable of penetrating and the germ tubes grow along the surface or into the air. The germ tubes appear to be negatively phototropic and

require the stimulus of light in order to orientate correctly prior to actual penetration.

The effect of reduced light intensity was studied on the field scale under the 7 foot atap shade. As mentioned previously there was a virtual absence of the disease under the shade, a transition zone of two or three yards at the edge, and heavy infection outside.

Artificial inoculations were carried out under the atap shade and events similar to those found in the case of laboratory inoculations were found to occur. The spores germinated, but only a proportion actually penetrated. Of those that penetrated none subsequently produced the typical spot, and further growth coincided with deposition of a barrier by the host cells. The resulting lesion appeared as a brown speck to the naked eye.

EFFECT OF DEW ON THE FUNGUS—For a number of plant pathogenic fungi, presence or absence of dew is a deciding factor in their successful germination and penetration. Dew provides a continuous film of moisture in which the spore can develop.

Laboratory studies on the physiology of germination have demonstrated the ease with which spores of *H.hevear* germinate in drops of distilled water. Germination in water drops placed on a susceptible leaf surface tends to be too vigorous, with repeated branching. Whilst the drop remains the fungus will not penetrate. The standard method of inoculation with a spore spray has been unsuccessful unless the drops are allowed to dry out before the germ tubes branch. So that it would appear that a continuously wet surface to the leaf constitutes an unfavourable condition for infection. A spore landing on the slightly damp surface of the young leaf normally produces a germ tube which penetrates without branching at all. As the dew is never of more than 6 to 8 hours in duration it will not prevent penetration, but may delay it by a few hours. But leaves under the shade, which are always free from a dew deposit, continuously provide a sufficiently moist surface for germination.

So far the influence of dew on the penetrating fungus has been discussed under the complication of a shading effect. It has been necessary to try to separate, experimentally, the two influences. On a small scale this has been done by growing plants under transparent screens. Bags of 'Alkathene' placed over whole flushes, and a frame of 'Windolite' 6 feet high, 4 feet long, and 2 feet wide, placed over whole plants, have been used. There was no deposition of dew, but the disease developed normally when spores were introduced into the enclosed space.

On a larger scale, and under ordinary field conditions, forty young seedlings were enclosed by a large frame, triangular in section, covered by roller blinds ('chicks') which could be raised or lowered at will. This structure was 6 feet wide at the base, 12 feet high from the ground to the ridge, and 12 feet long. The ends were covered with atap, and the faces with the blinds, 3 rolls 4 feet in width on each face. By lowering the blinds at sunset, and raising them at sunrise, dew formation was prevented, but other conditions were more or less

normal. After six months time measurements were taken of the 40 plants within the frame, and of a comparable block of 40 outside.

| | Plants inside frame | Plants outside frame |
|---|---|---|
| Mean height in inches .. | 47 | 40 |
| Mean diameter in mm .. | 10.7 | 8.7 |
| Mean no of leaves per plant .. | 21.3 | 17.6 |

This confirmed a visual impression that plants inside the frame were better grown than those outside. The plants inside showed a visible reduction in disease assessed as 'moderate', compared with 'heavy' outside. If dew was the determining factor one would have expected complete reduction of the disease; the leaves were wetted by rain, of course, but so were those on plants growing under the 7 foot shade. However if there were no shade, to what can this reduction be attributed ? The frame would certainly retard air movement at night time, and hinder access of spore bearing wind currents. Also the shading effect of the wooden frame, with its atap ends, cannot be discounted and is most likely responsible for the small reduction that does occur.

Summing up the effects of the shade upon the disease it appears that:

The factors additional to shading, acting as a protective screen against evaporation and against insolation, would be expected to favour fungus disease and it is therefore unusual to find the opposite effect.

The shade plant, being of very different anatomy and physiology from the sun plant, reacts rapidly against any spore that penetrates. It either contains an opposing factor or lacks some essential for growth. There are two very distinct forms of plant.

*Helminthosporium* can penetrate the damp susceptible leaves, despite the lack of standing water (*e.g.* from dew) that appears to be necessary for so many other fungi, but many spores fail to do so because of the lack of illumination. Table X suggests that illumination is necessary for proper orientation.

It thus appears that the presence or absence of dew is of no importance in the pathogenicity of *Helminthosporium heveae*, but the degree of illumination is very important, principally in its effect on the plant, but also in its effect on the fungus.

### Temperature

The vigour of this species of *Helminthosporium*, as shown by its rapidity of growth and its resistance to fungicides that are reported as effective against the genus in temperate climates, must be partly due to the favourable temperatures for fungus growth in the rubber growing areas.

Although records are lacking for incidence of the disease in the cooler rubber areas, some idea may be gained from the vigour of the parasite in culture of what its range in nature is likely to be. The temperature range in Malaya may be taken as 70°F (21°C) to 90°F (32°C). Temperatures a few degrees

above 32°C and below 21°C have been found to inhibit growth of the *Helminthosporium* colony in culture. It has not been possible to study the progress of infection on plants growing at these temperatures.

In certain other diseases temperature may have its influence on survival or germination of the spore of the pathogen but it has already (page 55) been shown that it exerts no effect on *H.heveae* within the range likely to be encountered in nature.

### Air movement

Studies on dissemination have demonstrated the importance of wind in spreading the disease from plant to plant and nursery to nursery.

## SOIL FACTORS IN THE ENVIRONMENT

With some of the obligate parasites, notably the cereal rusts, it has been shown that the more vigorous the plant, the more vigorous the parasite. By contrast it has often been demonstrated for other parasites that vigour of host and its proneness to disease may be antithetic variables. This appears to apply to some extent to *Helminthosporium heveae.* Ever since the disease became widespread about 1920 there has been a tradition that it is much less severe on good soils. On the rich volcanic soils of East Java, it is reported to be less troublesome than in West Java. West Java nurseries, in turn, are less severely attacked than those on the poorer soils of Sumatra. Finally Malaya seems to be more troubled with the disease than Sumatra.

Within Malaya the disease has been recognised as more severe on lighter and freely drained inland soils. Reports that the disease was virtually absent from coastal clays were found to be unfounded, though less defoliation and retardation seems to occur on these soils. Certain planters claim that they secure economic control of the disease by paying special attention to manuring their nurseries, and this does appear to be so. Reports have come from Honduras that applications of complete fertiliser have been very successful in securing economic control of the disease. The nitrogen source in this fertiliser is Chile saltpetre, not the synthetic ammonium sulphate used in Malaya. It has been suggested that minor elements present in Chile saltpetre may have an important effect on disease resistance.

No attempt has been made to separate the effects of nitrogen, phosphorus, and potassium applications. Experiments on these lines were carried out in Hawaii* on Eye-spot disease of sugar cane caused by *Helminthosporium sacchari*, and it was shown that with this species of *Helminthosporium* nitrogen applications during the winter months lead to a marked increase in infection, whilst potash applications resulted in a slightly lessened infection.

Drainage is a soil factor of some importance for other leaf diseases. On leaves of plants growing under badly drained conditions *Helminthosporium* is liable to appear, accompanied by many other fungi, giving the impression of an unusually severe

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* **Lee & Martin.** *Industr. Engng. Chem.* 20 (1928) 220

attack. 'Rim Blights' are a characteristic symptom of bad drainage.

It has been suggested that the resistance of shade leaves is connected with their high nitrogen content, and this in turn with more suitable conditions for nitrogen fixation under the shade. The heavy attack, as soon as leaves penetrated through the shade into the light, demonstrated that shade does not act through its effect on the soil. Mulching, too, which shades the soil and encourages microbial activity, has no effect in reducing the intensity of disease.

## BIOLOGICAL FACTORS IN THE ENVIRONMENT

### Plants

However favourable the physical environment, appearance of disease is dependent upon there being a source of infection. Normally this is from individuals of the same species, and the crowding of a large number of individuals into a small area occupied by one species, as occurs in cultivation of plants, invariably increases disease hazards.

Apart from the spread from plant to plant when once the disease has broken out, there is the question of the origin of the first outbreak in any area.

La Rue (1923) suggested that the source of infection was probably the leaves of the previous season. He suggests that under suitable conditions old leaves could easily infect young leaves of juvenile plants. He points out that differences in time of wintering would be sufficient to allow trees wintering late to infect those already bearing young flushes.

However 'wintering' in rubber does not present the same problems regarding survival of the fungus as true wintering in temperate climates. The rubber tree does not begin to 'winter' at all until it is three to four years old, and it is only on trees below this age that the disease is of any importance. There is ample material for the fungus to live parasitically all the year round. It has also been found experimentally that the fungus is able to grow saprophytically on dead vegetation in competition with other fungi. Thus La Rue's suggestion that the fungus survives on old leaves (although this doubtless could occur) is found to be unnecessary; nor need one postulate the existence of resistant fruiting stages.

Neighbouring plants of other species may be an important factor in the environment, either as competitors for food, light etc, or as harbourers of the pathogen.

As any estate nursery would be at least partially weeded, it is from the second point of view that other plants must be considered. The worldwide distribution of *H.heveae* makes it even more likely that it has other hosts.

As *Helminthosporium* is so prominently a graminicolous genus it is upon grasses that first suspicion falls. The following species of common grasses were examined, but without any evidence that they harbour *Helminthosporium heveae*.

Lalang, *Imperata cylindrica*, is a very widespread grass in Malaya, but is not invariably associated with the disease.

In Field 50 of the Experiment Station the disease practically disappeared during April and May 1949, but reappeared on re-invasion of weeds, of which the most prominent was *Tricholaena rosea*.

In the Sungei Buloh nursery at the time of the greatest build-up of the disease the grass *Panicum sarmentosum* was abundant. Other common grasses continuously present along the bunds were: *Ischaemum muticum*, *Paspalum conjugatum*, *Paspalum scorbiculatum*, and *Digitaria sanguinalis*.

Cultivated grasses could be responsible for harbouring the disease, and in the neighbourhood of a cultivated patch from which infection spread *Eleusine indica*, *Dactyloctenium aegyptium*, and *Saccharum officinarum* were examined.

Other Monocotyledons examined with the same negative results were *Caladium* sp. in view of a report from South India that *H.heveae* had been found on this plant; *Zingiber* sp, and *Commelina nudiflora*.

Palm trees support a vigorous growth of *Dematiaceae*, and a species of *Helminthosporium* morphologically very similar to *H.heveae* was isolated from oil palm. Rubber seedlings were inoculated with these spores and penetration took place, but no further development followed.

Some *Helminthosporium* species are known as parasites of Dicotyledons. First suspicion fell on the extremely common weed *Mikania scandens* which commonly bears spots with a brown margin and a clear central area covered with spores. The pathogen was shown to be a species of *Cercospora*, however.

Other weeds of the rubber nursery to come under suspicion were: *Borreria laevicaulis*, *Ludwigia parvifolia*, *Stachytarpheta indica*, *Gynandropsis gynandra*, *Portulacca oleracea*, *Jussiaea linifolia*, *Emilia sonchifolia*, *Oldenlandia corymbosa*, *Melochia corchorifolia* and *Lindernia crustacea*. No lesions bearing spores of a *Helminthosporium* have been found on any of these plants.

Amongst the *Euphorbiaceae*, *Euphorbia hirta* is a common weed of nurseries but it always appears to be free from disease, and inoculation with spores has not succeeded. Inoculations of *Manihot utilissima* (tapioca) and *Manihot glaziovii* (Ceara rubber) have also failed. La Rue (1923) had suggested that *Manihot utilissima*, or *Carica papaya* (papaw) might be the source of infection. The commonly occurring leaf spot of these species has been found to be due to fungi of the genus *Cercospora*.

### Animals

In many plant diseases animals play an important part in both dissemination and in bringing about infection. The earliest suggestion, made by Petch, was that *Helminthosporium* grew on spots made by insect punctures; since that time no further reference has been made in the literature to the actual mode of infection. A variety of insects and mites have been found on the rubber leaf but there is no indication that they contribute to *Helminthosporium* infection. No evidence has been found to support the suggestion that the fungus disease is connected with attack by the yellow tea mite *Hemitarsonemus latus*. Certain dipterous larvae were once found on the leaf with a variety of

dematiaceous spores, including *Helminthosporium*, in their gut but are not considered to have been of any importance.

Larger animals, and man most of all, can play an important part in dissemination by brushing against plants, so liberating spores into the air, and by moving from place to place carrying spores, leaves, or whole plants.

## VI. CONTROL

It has been seen that control can be effected by growing plants under shade, a method which might well be modified to be used under estate conditions. Complete control can also be obtained by protecting young leaves from the spores, but this is certainly impracticable for any but experimental purposes.

It has also been shown from the data and observations so far discussed that the *Helminthosporium* spore is unusually resistant to adverse conditions, and germinates and grows at a wide range of pH, and on a wide range of media. It is not surprising that this resistance also applies to fungicidal substances.

Preliminary trials with the usual field sprays and dusts showed them to be valueless in practice (Altson 1950*b*) and prompted a research into alternative fungicides which might be used in the field.

TABLE XI

*Fungicides to which the slide germination test has been applied*

| | Name of product employed | Active principle | Percentage Germination | |
|---|---|---|---|---|
| | | | in 0.001% active principle | in 0.0001% active principle |
| 1 | Spersul | Wettable sulphur | 100 | 100 |
| 2 | Standard laboratory Bordeaux mixture | Copper basic sulphates | 0 | 0 |
| 3 | Perenox | Cuprous oxide | 45 | 99 |
| 4 | Mercuric chloride | Mercuric chloride | 0 | 0 |
| 5 | Tersan | Tetramethylthiuram disulphide | 43 | 99 |
| 6 | Shirlan | Salicylanilide | 100 | 100 |
| 7 | Phygon | Dichloronaphthoquinone | 41 | 86 |
| 8 | Fermate | Ferric dimethyl dithiocarbamate | 89 | 91 |
| 9 | Zerlate | Zinc dimethyl dithiocarbamate | 50 | 90 |
| 10 | Dithane Z78 | Zinc ethylene bis dithiocarbamate | 40 | 98 |
| 11 | Malachite Green | Tetramethylaminotriphenylmethane | 51 | 95 |
| 12 | Fixanol V.R. | Tetradecyl Pyridinium bromide | 67 | 97 |

A rapid means of assessing such fungicides is the slide germination test, and a modification of this method was developed for use with *Helminthosporium*. It was found that production of a spore suspension was difficult as the spores produced in culture are comparatively small in number and difficult to obtain free from mycelium. Advantage was taken of the way in which the fungus spores heavily on lesions on the underside of the leaf, and spores were obtained directly from this source. After air drying for 12 hours the spore mass can be readily 'spattered' by means of a needle on to a 12 mm cover slip, similar to the way in which leaves are inoculated. The number of spores can be adjusted readily to the required number as they can be manipulated with a fine needle under the dissecting microscope. Allowance does not have to be made for the volume of the spore suspension when adding known volumes of fungicide at known concentration.

THE FUNGUS—This slide germination procedure is not, of course, a method using *Helminthosporium heveae* as a 'standard fungus' in the way that *Sclerotinia fructigena* and *Stemphylium sarcinaeforme* are used in the published standardized fungicidal tests. If it were so used conditions would have to be controlled with considerable care, and even then the results would not be comparable with standard tests, as these have always been carried out with a small number of standard species. Spores from a natural source were employed, to enable the fungicides to be tested under conditions as near as possible to those in the field. Stimulants were not added as *Helminthosporium heveae* invariably gave 100% germination in distilled water.

THE FUNGICIDE—Tests were confined to a range of readily available commercial products, chosen as representative of some of the various chemical groups of fungicides which have proved their worth against other pathogenic fungi, ranging from the old established sulphur to the recent quaternary ammonium derivatives.

It is confirmed from these results that sulphur, generally recommended at one time, is quite ineffective; this had already been indicated by field trials. Copper, however, is shown to be as promising as any of the newer fungicides. Comparison with published results of these fungicides on other species of fungi shows *Helminthosporium heveae* to be far less susceptible to fungicides, even in vitro, than many other fungi.

An account of field trials and the relation of field to laboratory results will be given in another Communication.

## SUMMARY

Bird's Eye Spot is a disease of young rubber plants first recorded by Ridley from Malaya in 1904, described by Petch from Ceylon in 1905, and named by Petch in 1906. It began to attract attention as a disease of rubber in Malaya in 1927. The disease

is widespread in the Asian, African, and American rubber-growing areas, there being few countries from which a record is lacking.

The importance of the disease is not in causing death of the plants, but in bringing about general weakening and defoliation. Consequently the plants take a longer time than they should to reach the optimum stage for transplanting or budding, with the result that planting programmes may be disrupted and the early development of new plantings seriously impaired.

The symptoms vary from a rampant infection accompanied by defoliation when it is the younger leaves that have been infected; typical 'bird's eyes' 2—3 mm in diameter infected when the leaf is fully expanded in the vertical position prior to hardening of the cuticle; to pin point spots when the leaf has been approaching the end of its susceptible phase at the time of infection.

The spores are large macroconidia, up to 200 $\mu$ long; no other conidial stage has been encountered, although chains of chlamydospores may be found in old cultures. No pycnidial or perithecial stage is known.

The spores germinate within 2 hours, and penetrate within 6 hours. They are highly resistant to heat and poisons. Both germinating spores and mycelium in culture tolerate a wide range of hydrogen ion concentration.

Inoculation of healthy leaves at the susceptible stage with spores from pure culture can be successfully undertaken and the pathogen subsequently recovered. The life cycle is completed in from 3 to 7 days, depending on the age of the leaf.

There is a stage of heavy sporing, usually occurring a few weeks after first infection of a plant, but also at other times under special circumstances. During most of the season sporing is relatively slight, although continuous. The heavy sporing stage is characterised by dark brown masses of spores on the underside of the leaf.

Dissemination is largely by wind, although rain, dew, blowing together of plants, and passage of labourers through the massed plants all contribute to freeing the spores from the lesions. Once the disease has appeared in a nursery its subsequent spread is very rapid and scarcely a single leaflet escapes infection.

Important factors governing the host-parasite relations are those of degree of shading and age of leaf. Growth of plants in sunlight, and immaturity of leaves must coincide if the disease is to develop, and different degrees of shade or age influence infection to the point when either heavy shade or a mature horizontal leaf prevent infection altogether.

No immune, or even resistant seedlings of *Hevea brasiliensis* have been encountered. *Hevea spruceana* and *H.brasiliensis* x *H.spruceana* hybrids are susceptible. *Hevea benthamiana* is resistant but not immune.

Environmental conditions likely to lead to epidemic attacks have been deduced from field records and from observations on

the life history. An epidemic would be expected after a period of dry weather on a poor soil with a large area of closely planted seedlings subjected to disturbance by wind or labourers.

The fungus has proved remarkably resistant to fungicides both under laboratory and field conditions. Sulphur has been found to be completely ineffective, copper fungicides more promising under certain conditions, but no economic method of fungicidal control can yet be claimed.

The text includes a review of all available literature on *Helminthosporium heveae* and these specific references to the fungus are grouped together in the complete bibliography following this summary. Passing references of a more general nature are included in the text as footnotes.

*The author wishes to express his indebtedness to Mr. R. A. Altson for suggesting the lines of this work, and for helpful advice and encouragement throughout.*

Pathological Division
The Rubber Research Institute of Malaya
Kuala Lumpur July 1952

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PLATE 1 Four-month old seedling showing 'sausage top' caused by swelling and defoliation of the upper portion of the stem. Note that the lower leaves, formed before the disease reached this part of the nursery, are free from infection.

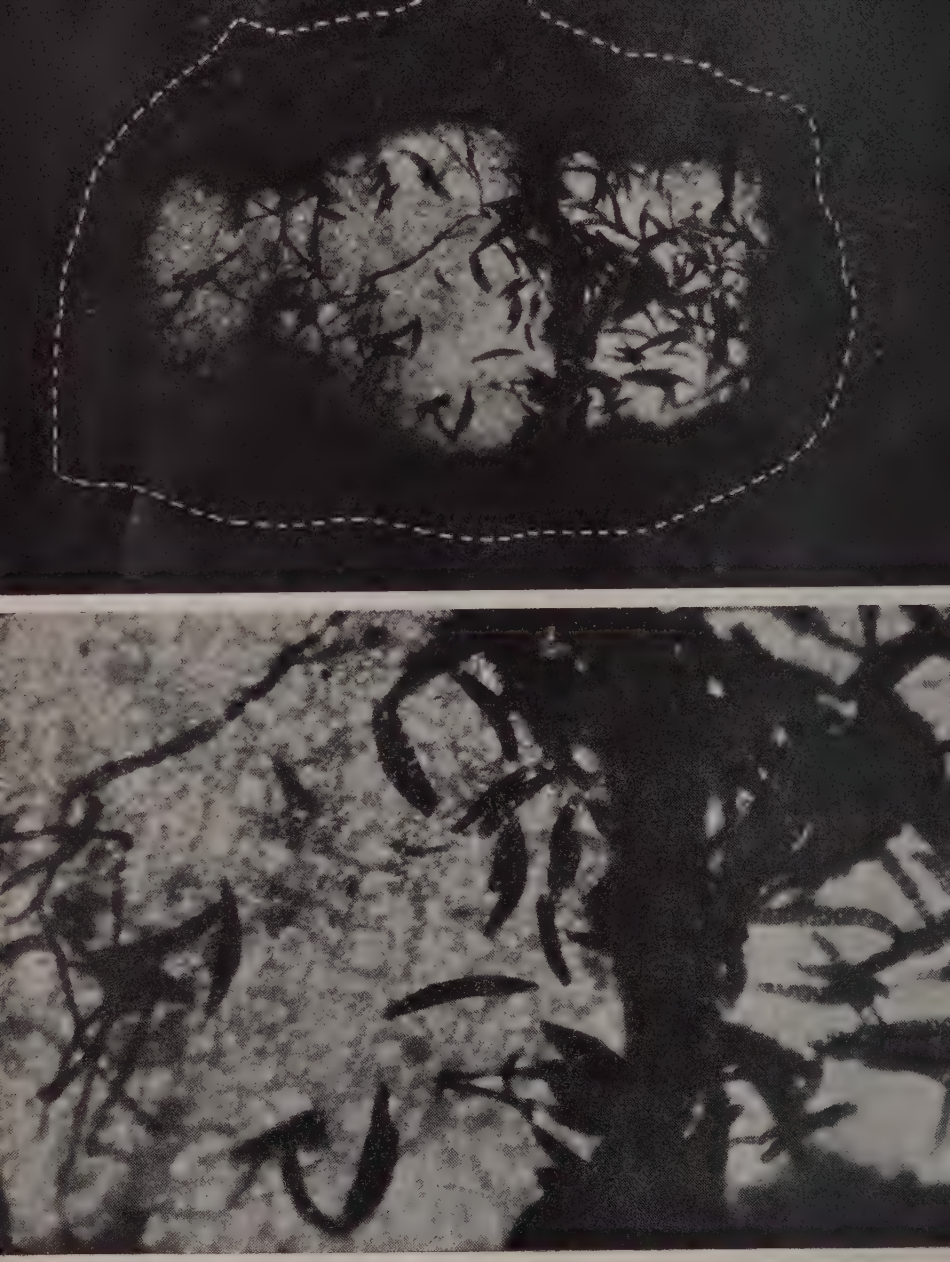
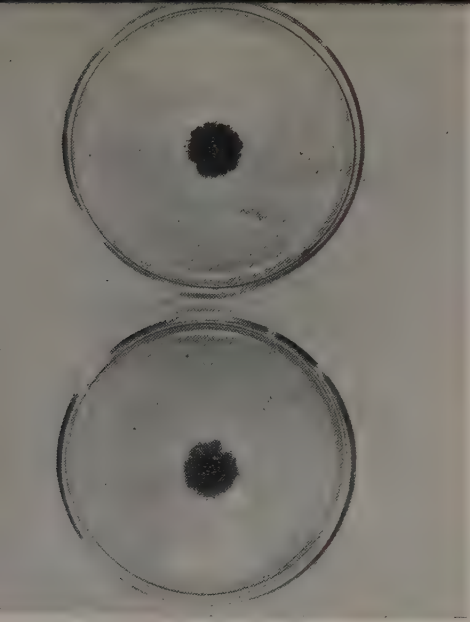


PLATE II Spores and conidiophores lying in their natural position on the surface of a lesion at the heavily sporing stage. A leaf vein and numerous stomata are visible in the clear central area. *Broken line indicates inner edge of brown margin.*



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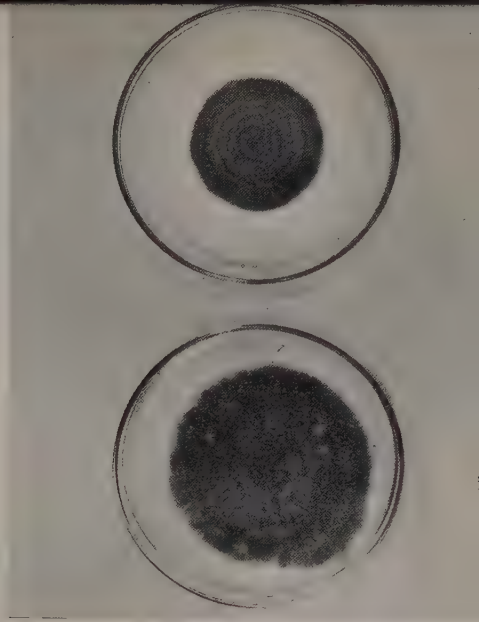
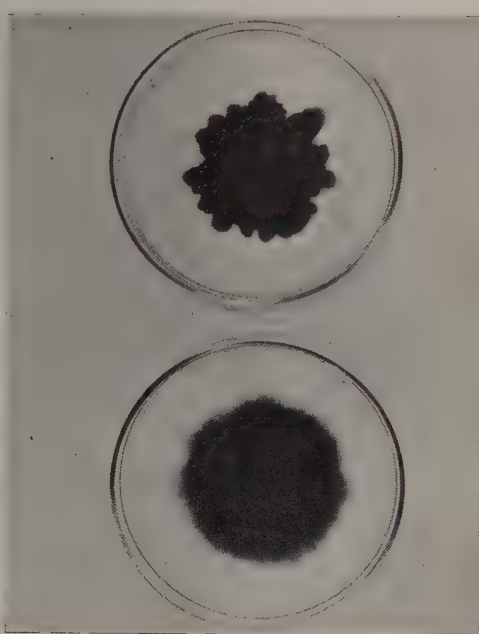


Potato Dextrose

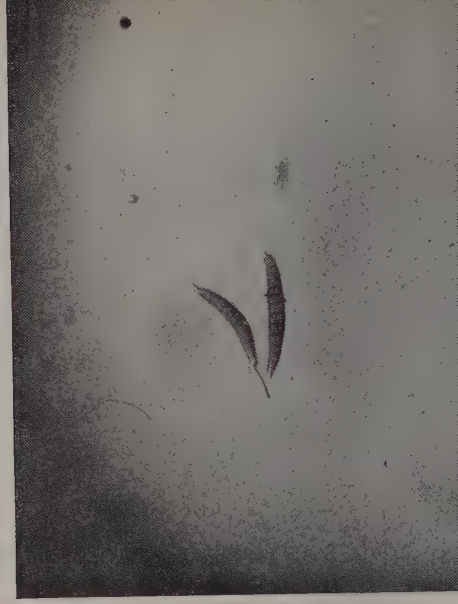
Corn Meal



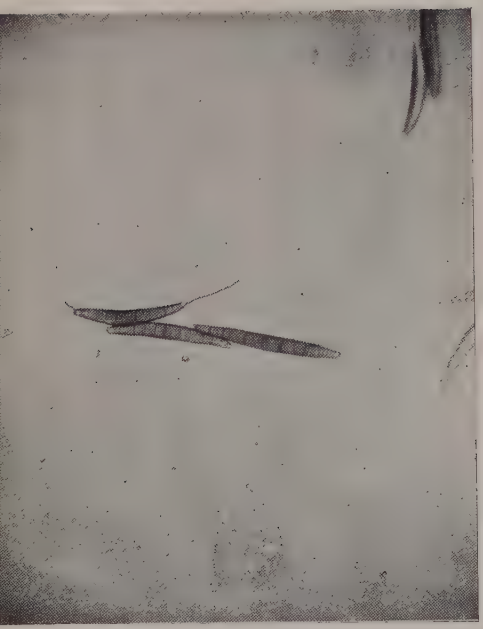
Dox

PLATE III Colonies after 7 days growth at room temperature showing zonation produced by alternating sporing and non-sporing bands. The upper dish of each pair has been grown under conditions of ordinary night and day lighting; the lower dish has been grown in the dark throughout.

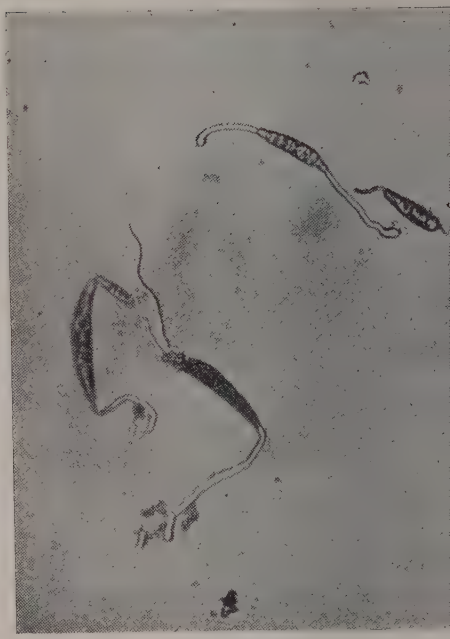
0900 hrs



1100 hrs



1300 hrs



1600 hrs

PLATE IV Germination of spores in distilled water

At time of Inoculation

2 days later

4 days later

6 days later

PLATE V Development of the lesion from a single spore inoculation

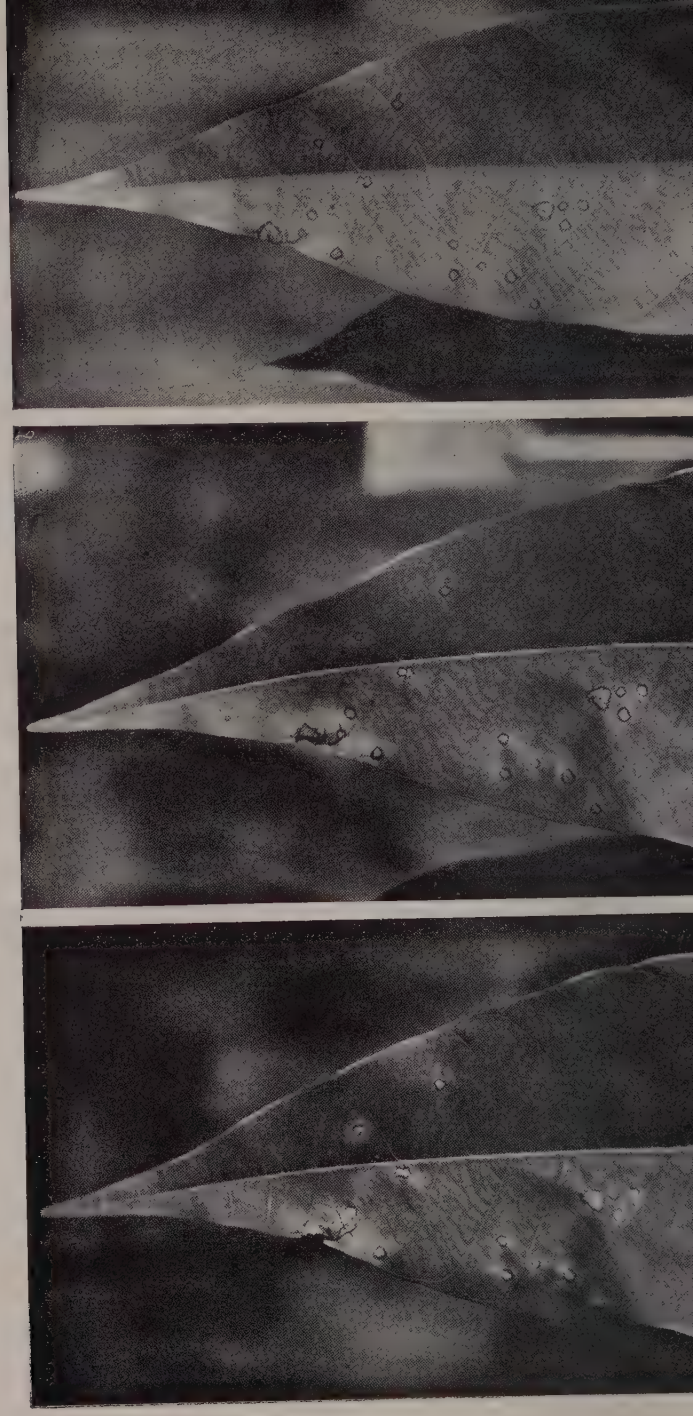



PLATE VI Development of the typical Bird's Eye Spot lesio

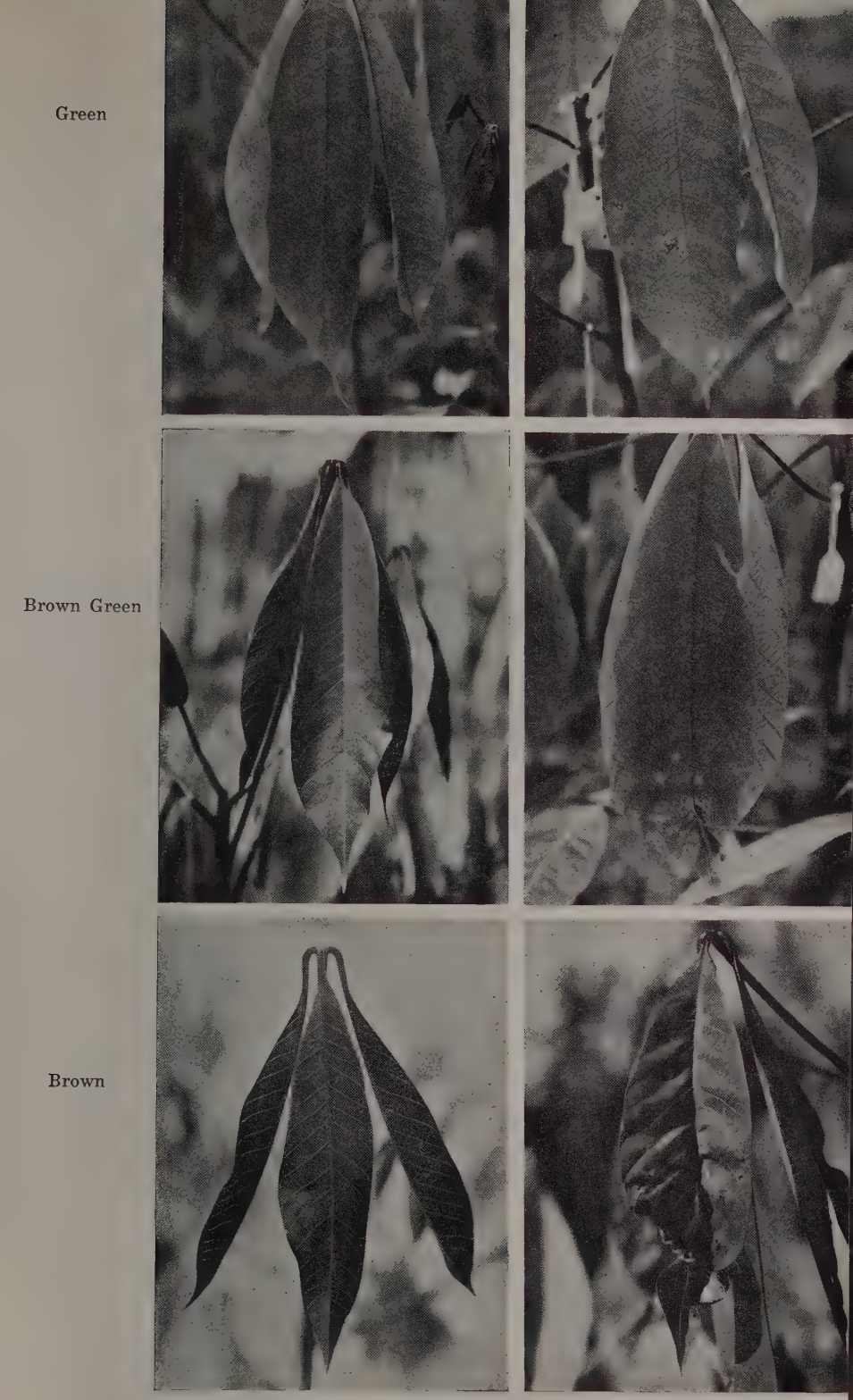



**PLATE VII** Leaves at green, brown green and brown stages respectively showing difference in type of lesion formed 5 days after inoculation.

PLATE VIII Budgraft showing shoot of scion (clone R.R.I. 619) virtually free from disease whilst the shoot of the seedling stock is heavily attacked.

PLATE IX Shade (on the left) and sun (on the right) leaves from the same plant showing the difference in colour, texture, and susceptibility to disease.

PLATE X General view of the ½ acre 7 ft atap shade at Sungei Buloh.

PLATE XI Seedling that has pierced the 7 ft atap shade, showing the first storey above the atap to be heavily attacked, whilst all storeys below are free from disease.

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